Proceedings of the 23rd Annual Aquatic Toxicity Workshop: October 7-9, 1996, Calgary, Alberta /

Comptes rendus du 23ieme atelier annuel sur la toxicité aquatique: du 7 au 9 octobre 1996, Calgary, Alberta

Editors/Éditeurs

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PREFACE

The 23rd Annual Aquatic Toxicity Workshop was held at the Palliser Hotel in Calgary, Alberta, October 7 to 9, 1996. The Workshop included 4 plenary presentations, 115 platform and 37 poster papers. Total attendance was 337.

The 23rd Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the Proceedings are published with the support of the Department of Fisheries and Oceans.

PREFACE

Le 23leme atelier annuel sur la toxicité a eu lieu L'Hôtel Palliser, Calgary, Alberta les 7 au 9 octobre 1996. Le atelier a donné lieu a 4 communications lors de séances plénières, 115 exposés d'invités d'honneur 37 communications par affichage. 337 personnes ont assisté au atelier.

Le 23leme atelier annuel sur la toxicité aquatique a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REMARQUES DES EDITEURS

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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TOXICITY TESTING FOR GAS PLANT DECOMMISSIONING

SESSION CHAIR: D.F. CHOLLAK

OKOTOKS SOUR GAS PLANT DECOMMISSIONING - SITE DESCRIPTION AND RECLAMATION PLANNING

D.F. Chollak. Canadian Occidental Petroleum Ltd., Calgary, AB.

The Canadian Occidental Petroleum Limited (COPL) sour gas plant is located east of the Town of Okotoks, AB. This plant was commissioned in 1959 and mothballed in 1991. Basic processes were to remove hydrogen sulphide gas from the 'sour' gas stream utilizing monoethanolamine (MEA) and to produce elemental sulphur, condensate and sales gas. Process wastes were deposited in on-site landfills and burial pits. Surface water runoff was directed towards a runoff lagoon while boiler blowdown wastes were sent to an adjacent lagoon. Hydrocarbon wastes were sent to one of two 'burn pits.' Process chemicals and various produced products were stored in tanks for later shipping by rail, truck or pipeline. Since shutdown in 1991, the plant has been subject to numerous soils, surface water, groundwater, vegetation and toxicity evaluations in support of detailed site assessments. These assessments culminated in CCME's National Classification System (NCS) for Contaminated Sites. Once this rating exercise was completed, it formed the basis for Human Health and Ecological Risk Assessment which will be used to determine 'risk-based' remediation criteria for the site. Toxicity evaluations are an integral part of the Risk Assessment. This paper will present an overview of both the historical plant operations as well as how the site assessments are used to develop preliminary remediation and reclamation plans.

SOIL AND VEGETATION STUDIES AT THE CANADIAN OCCIDENTAL OKOTOKS GAS PLANT SITE: USE IN PLANNING FOR REMEDIATION AND RECLAMATION ACTIVITIES

S.A. Leggett[1] and D.F. Chollak[2]. [1]3-D Reclamation Inc., Calgary, AB; [2]Canadian Occidental Petroleum Ltd., Calgary, AB.

In 1992, Phase I and II investigations were conducted at the Canadian Occidental Petroleum Ltd. Okotoks gas plant. The soils portion of the 1992 program consisted of 230 soil samples from 78 areas across the Upper and Lower Terrace. Sampling locations were selected based on the results of geophysical surveys and a review of plant historical and operating information. The purpose of the soil sampling program was to identify any contaminant issues present within the top one meter of soil and to provide information to determine the degree of risk associated with measured contaminants. In selected areas, such as the former landfill and amine pit areas, samples were taken to 3 meters. Vegetation samples were also taken from the Lower Terrace area that has been used for grazing and the toxicity potential of these samples was also assessed. Based on the results of the 1992 programs, additional soil, water and vegetation sampling programs have been conducted between 1993-1995 to fill in data gaps, delineate volumes of material that will require remediation and to obtain bulk samples for ongoing experimental and treatability studies. Results from the soil and vegetation sampling programs will be presented by principal areas within the plant site. These areas were established during the site rating study that was completed using the CCME National Classification System for Contaminated Sites.

HYDROGEOLOGY AND CONTAMINANT SITUATIONS AT THE OKOTOKS GAS PLANT

J.W. Fennell[1], J.H. Sevigny[1], T.L. Dabrowski[1] and D.F. Chollak[2]. [1]Komex International Ltd., Calgary, AB; [2]Canadian Occidental Petroleum Ltd., Calgary, AB.

The Okotoks Gas Plant is located on the Upper Terrace above the Sheep River. Drilling investigations have revealed that the Upper Terrace is composed of glacial till containing distinct groundwater-bearing zones. The Upper Terrace overlies sand and gravel deposits of the Sheep River flood plain, or Lower Terrace. Hydrogeologic evidence suggests that deposits at the base of the Upper Terrace are hydraulically connected to the Lower Terrace, and that groundwater flows southward towards the Sheep River valley. Sour gas was processed at the Okotoks Gas Plant from 1959 to 1991. Contaminant situations are evident in the process area and condensate storage area, downgradient of the burn pits, buried landfills, amine disposal pits, waste water ponds, and in the former sulphur storage area. Groundwater contamination is recognized by low dissolved oxygen values, anomalous indicator parameters, target and non-target hydrocarbons, and Microtox® response. Hydrocarbon characterization varies from good (i.e., condensate storage area) to poor (i.e., buried landfills and amine disposal pits). Groundwater contamination appears to be isolated to 'hotspots' on the Upper Terrace, and mainly restricted to the upper water-bearing zone. Lateral migration appears limited in most areas, with exception of the burn pits and waste water ponds. Hydrochemical evidence collected to date suggests mass loss of some organic contaminants via intrinsic bioremediation.

RESULTS OF TOXICITY TESTING USING SOIL AND GROUNDWATER SAMPLES COLLECTED FROM THE CANADIAN OCCIDENTAL PETROLEUM LTD., OKOTOKS, ALBERTA GAS PLANT

J.S. Goudey[1], R.D. Shaw[2], H.R. Hamilton[2], D.F. Chollak[3] and J.G. Ward[4]. [1]HydroQual Laboratories Ltd., Calgary, AB; [2]Golder Associates Ltd., Calgary, AB; [3]Canadian Occidental Petroleum Ltd., Calgary, AB; [4]Amoco Canada Petroleum Co. Ltd., Calgary, AB.

Canadian Occidental Petroleum Limited is reclaiming a gas plant at Okotoks, AB. Chemical and toxicological analyses were to used to determine the extent of contamination at the site. During Phase I, chemical analyses were performed on whole samples and screening level toxicity tests were performed on order-of-magnitude dilutions of soils, groundwaters and leachates. In general, soil samples were more toxic than groundwater samples. Of the samples analyzed in this study, the MEA pits had the highest overall toxicity rating. Next, in descending order, were samples from the

condensate area, sulphur base pad, utilities lagoon, burn pit and process building areas. Phase II used soils from three most highly toxic areas on the study site to develop and test a method for performing a Toxicity Identification and Evaluation procedure on solid-phase samples. Solid-phase toxicity in the sulphur base pad sample and the MEA pits sample was removed by one or two water extractions, but two water and two methanol extractions were necessary to remove toxicity in the condensate area sample. The extractions identified salts as important sources of the observed toxicity at all three sites, along with petroleum hydrocarbons (particularly sulphur heterocycles) at the condensate area, MEA and breakdown products at the MEA pits, and unidentified sulphur compounds at the sulphur base pad. The results of these studies along with those from supplemental biological and toxicological sampling conducted in the summer of 1996, Phase III, will be discussed with respect to ecological risks associated with this site.

A COMPARISON OF PRELIMINARY RISK ASSESSMENT RESULTS TO CCME NATIONAL CLASSIFICATION SYSTEM RESULTS AT THE OKOTOKS GAS PLANT

G.L. Brown[1], R.M. Wilson[1], G.A. Clyde[1] and D.F. Chollak[2]. [1]CanTox Inc., Calgary, AB; [2]Canadian Occidental Petroleum Ltd., Calgary, AB.

The Okotoks sour gas processing plant site was initially assessed according to the Canadian Council of Ministers of the Environment (CCME) National Classification System for Contaminated Sites (NCS) (CCME, 1992). Following CCME site classification, which identified the potential need for remediation at specific locations within the site, a preliminary human health and ecological risk assessment was completed for the Okotoks site. The objectives of the preliminary risk assessment were to provide screening level information regarding potential risks to human and ecological receptors based on existing information, and to identify any additional data needed to complete the risk assessment. This work is part of an ongoing program that will ultimately result in generation of risk-based remediation criteria for the site. The risk assessment was based on the existing environmental sampling and toxicity testing that has been collected by other consultants at the site since 1987. For the human health risk assessment, two potential land use scenarios were considered including industrial use and parkland use. The preliminary ecological risk assessment involved synthesis of existing sampling and toxicity testing results, as well as prospective assessment of potential risks to ecological receptors of concern (meadow vole and red-tailed hawk). The steps in the preliminary risk assessment included chemical screening, receptor and exposure pathway selection, toxicity assessment of chemicals of concern, estimation of exposures, and risk characterization. The results from both the CCME NCS classification and from the preliminary human health and ecological risk assessment for the Okotoks site will be presented and compared.

ADDRESSING REGULATORY ISSUES USING TOXICITY TESTING AND FATE ASSESSMENT OF CONTAMINANTS IN GROUND WATER

SESSION CHAIR: J.H. SEVIGNY

CHEMICAL CONTAMINATION OF GROUNDWATER AT GAS PROCESSING PLANTS - THE PROBLEM

R.M. Wrubleski and C.R. Drury. Shell Canada Ltd., Calgary, AB.

Over the history of natural gas processing in western Canada, sulfur compounds have been removed from 'sour' gas in processes using various chemical solvents. The chemicals, primarily amines and

physical absorbents, have ended up as contaminants in soil and groundwater at a number of sites. This project focuses on groundwater contamination by Sulfolane and Disopropanolamine (DIPA) which originate from the Sulfinol process. The scientific procedures developed can also be applied to other chemicals such as the conventional amines and glycols. Sulfolane and DIPA contamination in the subsurface around gas plants is related to generally known sources (spills and old landfills). However, information is very limited regarding transport phenomena, long-term fate (attenuation, biodegradability) and ecological and human health effects. Such information is required to properly model subsurface behaviour, establish risk-based clean-up criteria and the need for any remedial measures. A research program on fate and toxicity assessment was identified to obtain the required data.

A REGULATORY PERSPECTIVE ON THE USE AND APPLICATION OF SHORT-TERM TOXICITY TESTS FOR ASSESSMENT OF CONTAMINATED SITES

M. Zemanek and G. Dinwoodie. Alberta Environmental Protection, Contaminated Sites and Decommissioning Branch, Edmonton, AB.

The overtly toxic nature of some chemicals released to the environment has prompted regulatory authorities to set generic remediation criteria for contaminated sites. These normally consist of specified concentrations of individual chemicals in soil, air or water and provide a conservative basis for the limits of site remediation and future land use. While target chemical analysis of an impacted soil or water is a fundamental step for characterizing the origin, nature and degree of contamination, it may not fully address the biological effects that a complex contaminant mixture may have on terrestrial and aquatic organisms. Even if every chemical concentration in a complex mixture could be successfully quantified, published criteria, toxicity databases and models for prediction of ecological effects are limited and do not explicitly address interactive effects. Short-term toxicity tests (acute and chronic), when applied in conjunction with chemical analysis, can be used to overcome some of these deficiencies and uncertainties when evaluating chemical impacts on the environment. Short-term toxicity tests performed on ecologically relevant organisms are a useful tool when applied in support of ecological risk assessments because they can provide [1] an improved understanding of potential adverse effects of chemical mixtures on biota, [2] site-specific toxic responses that may not otherwise be predicted by published toxicological data, [3] a better understanding of toxic effects of chemicals for which there is no or limited published toxicological data, [4] an aid in the identification of individual or classes of toxic chemicals to refine subsequent analytical identification and mitigative efforts (TIE evaluation), and [5] measurable environmental characteristics (measurement endpoints) which quantify impacts in an ecological risk assessment. This presentation will provide some insight as to regulatory expectations for the use of toxicity testing within an ecological framework.

LABORATORY STUDIES TO ASSESS THE FATE OF SULFOLANE AND DIISOPROPANOLAMINE IN THE SUBSURFACE AT SOUR GAS PROCESSING PLANT SITES

P.M. Fedorak, D.L. Coy, L.M. Gieg, E.A. Greene, S.M. Luther and M.J. Dudas. University of Alberta, Departments of Biological Sciences and Renewable Resources, Edmonton, AB.

Diisopropanolamine (DIPA) and sulfolane are water-soluble compounds used to remove H_2S from sour gas. Spills and landfilling have introduced these compounds into soil and groundwater at sour gas plants. The environmental fate of these compounds depends in part on their biodegradability and their transport through soil and groundwater. An ongoing project is assessing the biodegradability and the sorption of DIPA and sulfolane. Sediments from contaminated aquifers have been tested for the presence of microorganisms that are able to degrade these two compounds under aerobic and

anaerobic conditions. Individual anaerobic microcosms containing nitrate, Mn(IV), Fe(III), sulfate or CO_2 as terminal electron acceptors were inoculated with contaminated sediments, and incubated at 28° and 8°C. Preliminary results indicate that sulfolane is more resistant to anaerobic degradation than DIPA. In contrast, sulfolane and DIPA were readily degraded under aerobic conditions in shake-flask cultures. Sulfolane serves as the sole carbon and sulfur source for enrichment cultures, and release of sulfur as sulfate was observed. Similarly, DIPA serves as the sole carbon and nitrogen source for enrichment cultures, and isopropanolamine was observed as a transient intermediate of DIPA degradation. Sorption studies with various soils indicated that the distribution coefficients (K_d) for sulfolane are quite low (≤1 L/kg), whereas K_d values for DIPA are somewhat higher. These results suggest that sulfolane will be less attenuated than DIPA in groundwaters.

THE TOXICITY OF SULFOLANE AND DIPA GAS PROCESSING PLANT CONTAMINANTS TO AQUATIC SPECIES

D.R. Lintott[1], J.S. Goudey[1], J. Wilson[2] and S. Swanson[2]. [1]HydroQual Laboratories Ltd., Calgary, AB; [2]Golder Associates Ltd., Calgary, AB.

Sulfur compounds are removed from 'sour' gas during natural gas processing using various chemical solvents. Contaminants from these processes have been found in soil and groundwater at a number of sites in Western Canada. The ecotoxicological effects of sulfolane and diisopropanolamine (DIPA), and their process related and biodegradation breakdown products are unknown. A study was initiated to characterize the toxicity of these materials to organisms representative of major trophic levels in aquatic systems. The test species included microbes (bacterial luminescence), plants (*Selenastrum capricornutum*), invertebrates (*Ceriodaphnia dubia, Daphnia magna*) and fish (*Pimephales promelas*). The concentrations at which sulfolane and DIPA exhibited toxicity are considered high relative to many other chemicals of environmental concern. Several lots of technical grade materials were compared to reagent grade results to evaluate effects of potential contaminants. The toxicity of microbial degradation products suggest that clean-up by bioremediation would not pose a risk due to toxic metabolites. Evaluation of reclaimer bottoms by toxicity mass balancing has indicated the presence of sulfolane and DIPA presents a toxicity component attributable to these chemicals.

NATURE, EXTENT, AND TOXICITY OF SULFOLANE AND DIPA CONTAMINATED GROUNDWATER: IMPLICATIONS FOR INTRINSIC REMEDIATION AT A SOUR GAS PLANT SITE

J.H. Sevigny[1] and R.M. Wrubleski[2]. [1]Komex International Ltd., Calgary, AB; [2]Shell Canada Ltd., Calgary, AB.

Leachate from poorly constructed landfills has contaminated groundwater at a number of sour gas plants in western Canada. Contaminated groundwater discharge from springs or into creeks occurs at some sites. Thus, potential human health or ecological exposure pathways exist. We have studied the nature, extent, and toxicity of sulfolane and diisopropanolamine (DIPA) contaminated groundwater at a sour gas plant in southern Alberta to evaluate intrinsic remediation potential under aerobic and anaerobic conditions and support risk-based corrective action measures. The landfill cells contain a variety of wastes including sulfolane and DIPA contaminated materials. Groundwater moves at a velocity of 5 to 30 m/yr through partially submerged landfill cells. Downgradient piezometer samples have high concentrations of chloride, dissolved organic carbon, Kjeldahl nitrogen, sulfolane, DIPA and carboxylic acids. The groundwater samples also show a significant response to Microtox®. Aerobic activity is expected at plume boundaries, and the presence of manganese and iron introduce the potential for anaerobic activity in the oxygen deficient zones. Significant findings of these data are [1] behaviour of DIPA relative to sulfolane, [2] mass balance of dissolved carbon and organic nitrogen with sulfolane and DIPA, and [3] toxicity correlated with sulfolane and DIPA concentration.

TOXICITY TESTING FOR ECOLOGICAL RISK ASSESSMENT IN THE PETROLEUM INDUSTRY

SESSION CHAIR: L. CALDER

DECOMMISSIONING, REMEDIATION AND RECLAMATION OF A FORMER OIL REFINERY SITE IN CALGARY, ALBERTA

D. Gorling. Thurber Environmental Consultants Ltd., Calgary, AB.

An oil refinery operated in the community of Inglewood, just east of downtown Calgary, from the 1930's until approximately 1985, when decommissioning of the facility began. Subsurface groundwater contamination first became evident in 1977 when an oil slick appeared on a lagoon at the adjacent Inglewood Bird Sanctuary. This discovery prompted an extensive soil and groundwater investigation to define the extent of subsurface hydrocarbon contamination in the vicinity of the refinery site. Several liquid hydrocarbon recovery systems were installed to recover oil from the groundwater surface. It is estimated that the total volume of liquid hydrocarbon recovered from the subsurface is in the order of 1.5 million litres, which has been gradually declining with time. The refinery site has recently been transformed into Wildlands Park. Much of the contaminated surficial soil, which was removed as part of the refinery's decommissioning, has been replaced with imported soil. The surface of the site has since been recontoured and revegetated with a wide variety of trees, shrubs and ground cover. An ornamental pond has been constructed in the centre of the site, which incorporates an engineered clay liner to minimize its potential impact on the underlying groundwater contaminant plume. Groundwater monitoring and management will continue in order to minimize the potential for migration of the liquid hydrocarbon plum which remains beneath the site.

CHEMICAL AND BIOLOGICAL EVALUATION OF PETROLEUM CONTAMINATED SOIL BIOTREATMENT FACILITIES IN THE ATLANTIC REGION

M. Dober[1], J. Carter[2], L. Steward[3], K. Doe[1], K.L. Tay[1] and J.S. Goudey[4]. [1]Environment Canada, Dartmouth, NS; [2]Carter Environmental Services, Halifax, NS; [3]New Brunswick Department of Environment, Fredericton, NB; [4]HydroQual Laboratories Ltd., Calgary, AB.

Soil contaminated with petroleum products due to accidental release represents a significant proportion of the contaminated material requiring remediation in Atlantic Canada. The number of provincially-licensed treatment facilities established in the Region is growing, particularly in New Brunswick. Such facilities utilize either thermal technologies, such as low temperature thermal desorption, or biological methods, which include landfarming and ex-situ bioremediation.

Ex-situ bioremediation is widely used for the treatment of petroleum contaminated soils because of its relatively low operating costs and the fact that most petroleum products are amenable to biological degradation. Depending on the facility, biopiles are supplemented with varying levels of nutrients, aeration and bioculture to enhance the degradation process and are monitored regularly. Once acceptable TPH levels have been achieved, the treated soil is removed from the site.

In order to evaluate the effectiveness of bioremediation facilities in the Atlantic Region, Environment Canada conducted a study of two New Brunswick facilities in cooperation with the New Brunswick Department of Environment (NBDOE). Untreated and treated soils were evaluated using chemical and biological assessment methods to determine if contaminants in the soil and any toxicity to test organisms have been reduced through treatment to levels acceptable for its end use. Treated and

untreated soils were analyzed for moisture, pH, grain size, TPH, PAHs, metals, nutrients, PCBs, and a variety of bacterial counts (total bacteria and hydrocarbon degraders). In addition, samples were subjected to a battery of toxicity tests which included solid phase tests (earthworm, lettuce seedling emergence, and Microtox® solid phase test) and liquid extract tests (water and methanol extracts tested using the *Selanastrum capricornutum* growth test, the Microtox® test, and the lettuce seed root elongation test). These results will be discussed and recommendations for future testing will be provided.

SENSITIVITY OF SCREENING-LEVEL TOXICITY TESTS USING SOILS FROM A FORMER PETROLEUM REFINERY

S. Pauwels[1], J. Bureau[2], Y. Roy[2], B. Allen[3], P. Y. Robidoux[3], M. Soucy[3] and N. Marchand[4]. [1]Exxon Biomedical Sciences, Inc., NJ; [2]Analex, Inc., Montreal, QC; [3]ADS, Montreal, QC; [4]Imperial Oil Ltd., Montreal, QC.

We tested five composite soil samples from a former refinery. The samples included a reference soil (Mineral Oil and Grease, MO&G <400 mg/kg), thermally-treated soil (MO&G <40 mg/kg), biotreated soil (MO&G = 3,100 mg/kg), and two untreated soils (No. 1, MO&G = 2,000 mg/kg and No. 2, MO&G = 6,900 mg/kg). We evaluated toxicity using the earthworm *Eisenia fetida*, lettuce, cress, barley, Microtox®, green algae, fathead minnow, and *Daphnia magna*. The endpoints measured were lethality, seed germination, root elongation, growth, and bioluminescence. Toxicity, as measured by the number of positive responses, increased as follows: biotreated soil <untreated soil No. 1 <reference soil <thermally-treated soil and untreated soil No. 2. The biotreated soil only had one positive response, whereas the thermally-treated soil and untreated soil No. 2 had five positive responses. The most sensitive and discriminant terrestrial endpoint was lettuce root elongation which responded to untreated soil No. 1, thermally-treated soil, and reference soil. The least sensitive was barley seed germination for which no toxicity was detected. The most sensitive and discriminant aquatic endpoint was green algae growth which responded to untreated soil No. 1., thermally-treated soil, and reference soil. The least sensitive was *D. magna* for which no toxicity was detected. Overall, soil and aqueous extract toxicity was spotty and no consistent patterns emerged to differentiate the five soils. Biotreatment significantly reduced the effects of the contamination. Aqueous toxicity was measured in the reference soil, probably because of the presence of unknown dissolved compounds in the aqueous extract. Finally, clear differences in sensitivity existed among the test species.

INCORPORATION OF TOXICITY TESTING IN SITE ASSESSMENT AND REMEDIATION

D.A. Birkholz[1], R. Rogers[2], J.S. Goudey[3], J.H. Sevigny[4], L. Calder[5] and B. Mersereau[6]. [1]Enviro-Test Laboratories, Edmonton, AB; [2]Toxcon Inc., Edmonton, AB; [3]HydroQual Laboratories Ltd., Calgary, AB; [4]Komex International Ltd., Calgary, AB; [5]Shell Canada Products Ltd., Calgary, AB; [6]Canadian Turbo Inc., Calgary, AB.

Site remediation for contaminated sites may be conducted following chemical analyses of soil and groundwater for specified chemical parameters. These parameters are defined by regulatory agencies and include analyses for metals, mono-, polyaromatic-, and chlorinated hydrocarbons, phenolic and miscellaneous organic and inorganic compounds. Following analyses, data are scored and regulatory exceedences noted. A site remediation plan may be developed using regulatory guideline criteria (chemical specific endpoints) or a risk assessment. From an environmental protection point of view such an approach raises a number of concerns. A limitation of criteria-based approaches revolves around the fact that the number of pollutants identified by the regulatory agencies is minuscule

compared to the number of known chemicals. Therefore cleanup to chemical-specific endpoints raises questions as to the presence of toxic chemicals which may not be listed by regulatory agencies and which should be included in a site remediation plan.

An alternative to the regulatory chemical-specific approach is to use a toxicity-based approach. In addition to collecting regulatory agency chemical-specific data for contaminated groundwater samples from a contaminated site, toxicity identification evaluations were performed. Chemicals of concern identified using the chemical-specific approach were monoaromatic hydrocarbons, whereas the toxicity based approach revealed that the major contributor to toxicity was *t*-butylphenol. Follow up investigations revealed that the *t*-butylphenol was part of an operation which had been missed during the Phase I audit of the site. As such the TIE is a powerful supplemental tool for site remediation at complex sites, as it is capable of detecting chemicals contributing to toxicity but not listed by the regulatory agencies.

DEVELOPMENT OF HUMAN HEALTH-BASED REMEDIATION CRITERIA FOR TOTAL PETROLEUM HYDROCARBON (TPH)

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The contamination of soils and groundwater by petroleum products is a problem commonly encountered in remediation of petroleum-associated industrial sites. Petroleum products can be released into the environment from various types of production, distribution, and storage facilities due primarily to accidental leaks and spills. Chemical analyses of petroleum products, mainly fresh, and to some extent, weathered, demonstrate they are composed of a large number of chemicals that differ in toxicity, physical-chemical properties, environmental fate, and concentrations within various products. To characterize the extent of contamination at a site with petroleum products, a total petroleum hydrocarbon (TPH) analysis is commonly used to indicate the magnitude of contamination. However, there are limitations to the use of the TPH parameter for site characterization, in that the chemical composition of the mixture is not indicated, and a link to toxic impact cannot be made. Therefore, it cannot be used for either the assessment of potential human health risks associated with a site, or for the development of health-based remediation criteria for various environmental media at a site. An approach has been developed that would enable the use of the TPH parameter, together with chemical-specific toxicological and analytical data, in the assessment of potential adverse health (human and/or ecological) impacts from a petroleum-contaminated site, and in the development of site-specific remediation objectives. The approach retains the advantages of the TPH parameter as an indicator of petroleum hydrocarbon contamination (simplicity of measurement, reasonable cost and measure of entire chemical mixture), while providing a linkage to the toxicity of the various chemical components of specific TPH measurements.

In our approach, the potential constituents of TPH were identified via a review of the literature regarding chemical analyses of fresh petroleum products, as well as through unpublished data from analyses of TPH at petroleum-contaminated sites. Based on physical-chemical properties, and available toxicological data, these constituents were assigned to groupings of related compounds. Candidates for a representative "surrogate" chemical for each group were selected based on the availability of reliable toxicity data suitable for the derivation of an exposure limit. In cases where data were sufficient to derive exposure limits for more than one of the group members, the chemical with the most complete data or with the greatest toxic potency was selected to be the 'surrogate.' The toxicities of the other chemicals within the groups were incorporated via the use of toxic potency factors, which related their toxicities to that of their respective 'surrogates.' The toxic potencies of compounds lacking sufficient data for the development of exposure limits were assigned a potency factor of 1.0 relative to the

'surrogate.' The physical-chemical properties, environmental fate and pharmacodynamics of the members of a group were assumed to be the same as those of the 'surrogate.' The groups of chemicals and their assigned 'surrogate' selected for this TPH method includes butane (C_1 to C_4 alkanes and alkenes), hexane (C_5 to C_8 alkanes and alkenes), nonane (C_9 to C_{18} alkanes and alkenes), eicosane (C_{19} to C_{35} alkanes and alkenes), cyclohexane (cycloalkanes and cycloalkenes), benzene, ethylbenzene (substituted benzenes), benzofuran (substituted benzofurans), benzo[a]pyrene (carcinogenic PAHs), and anthracene, naphthalene and phenanthrene (for non-carcinogenic PAHs, divided based on physical-chemical properties).

In the application of this TPH method to deriving risk-based remediation criteria, a site-specific characterization of the TPH is based on detailed chemical analyses of a minimal number of representative samples from the site, in conjunction with a more comprehensive sampling for determination of TPH concentrations alone around the site. A toxicity-adjusted concentration is calculated by multiplying the measured concentration of each group member by its toxic potency factor, then summing these 'surrogate'-equivalent concentrations for all members of each group. A ratio between this summed 'surrogate' concentration and the TPH concentration at the same location is then calculated. The 'surrogate'/TPH ratio is then applied to the various locations on the specific site where only TPH measurements have been determined to estimate the concentrations of the 'surrogate' chemicals for each of the TPH sampling locations on the site. The potential risks from the TPH contamination of the site can then be estimated by comparisons between the expected exposures of selected receptors, based on the 'surrogate'-equivalent concentrations at the site, through various critical exposure pathways, and the exposure limits derived based on the toxic potency of each of the 'surrogates.'

In conclusion, our TPH risk assessment method for protection of human health effects is similar in concept to that recommended by the Massachusetts Department of Environmental Protection and currently being developed by the TPH Criteria Working Group. Our method enables the use of the TPH parameter, together with toxicological and analytical data, in the assessment of potential adverse health impacts from the petroleum-contaminated sites, and in the development of site-specific remediation criteria. This approach retains the advantages of the TPH parameter as an indicator of petroleum hydrocarbon contamination, while providing a linkage to the toxicity and environmental fate of various chemical components of each TPH source.

OIL SANDS

SESSION CO-CHAIRS: R.D. SHAW, J.R. GULLY and M.D. MacKINNON

OIL SANDS DEVELOPMENT - OPERATIONAL REALITIES AND ENVIRONMENTAL ISSUES

J.R. Gulley. Golder Associates Ltd., Calgary, AB.

Development of the open pit mining operations for recovery of oil resources from oil sands began in the mid 1960's. The extraction and processing of oil sands for production of synthetic crude oil has resulted in a number of environmental issues, including [1] significant areas of land disturbance, [2] generation of enormous volumes of tailings and fine tails (clay-water suspensions), and [3] mobilization of chemicals associated with the bitumen (heavy oil) within the oil sands. Two companies, Suncor Inc., Oil Sands Group and Syncrude Canada Ltd., have developed full-scale oil sands operations near Fort McMurray, AB. Both companies have conducted extensive engineering and environmental research and development programs over the past couple of decades to address operational and environmental

issues. The past and current research efforts have been coordinated through multi-disciplinary alignments among industry, government, universities and independent research organizations. Two such alignments, the Fine Tailings Fundamentals Consortium and the Canadian Oil Sands Network for Research and Development (CONRAD), have functioned to assist in focusing research efforts and maximizing the impact of monies invested in resolution of issues associated with oil sands development. The results of the oil sands research efforts have allowed the operating companies to modify their operational processes and improve their reclamation programs to enhance the return of the disturbed areas back to alternate end uses. The results of the research have also allowed focusing of current and future research efforts to resolution of specific remaining issues.

BASELINE AQUATIC OVERVIEW - AQUATIC BIOTA IN THE OIL SANDS AREA

M. Lagimodiere, L. Gummer, K. Holley, Z. Kovats and R.D. Shaw. Golder Associates Ltd., Calgary, AB.

Suncor Inc., Oil Sands Group (Suncor) and Syncrude Canada Ltd. (Syncrude) currently operate oil sands facilities on the west side of the Athabasca River, north of Fort McMurray. Both companies plan to expand their operations, Suncor in the vicinity of the Steepbank and Athabasca Rivers (Steepbank Mine) and Syncrude in the vicinity of the Muskeg River (Aurora Mine). As part of the environmental impact assessments, an aquatic study was conducted in 1995 to describe the current conditions with respect to surface water, porewater and sediment quality, benthic invertebrates, fish habitat, fish communities, and fish health, and provide a baseline for comparing future conditions. The study area included portions of the Athabasca, Steepbank and Muskeg Rivers as well as several tributaries. Naturally-occurring hydrocarbons can be found in river sediments and porewater, however, no changes in surface water chemistry are associated with Athabasca oil sands deposits or existing oil sands facilities. Benthic invertebrate communities are thriving and show no evidence of negative effects associated with exposure to naturally occurring hydrocarbon deposits or existing oil sands developments. Fish habitat in the Athabasca River within the study area is relatively poor because of the homogeneous habitat and shifting sand bottom. High quality habitat exists in the Steepbank and Muskeg Rivers and in some tributaries to these rivers. There are diverse fish communities in the Athabasca, Steepbank and Muskeg River basins. There is evidence of exposure of fish to naturally-occurring hydrocarbons, although fish general fitness and health indicators suggest that fish populations are healthy.

HYDROLOGY AND HYDROGEOLOGY IN THE OIL SANDS AREA

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The hydrology and hydrogeologic setting of the Alberta Oil Sands area near the Suncor Inc. oil sands operation north of Fort McMurray, AB, was studied in detail as part of the recently completed Environmental Impact Statement for the proposed Steepbank Mine. This paper presents a summary of the hydrology, geology, geochemistry and hydrochemistry in a relatively undisturbed area of the Oil Sands as they relate to the aquatic habitats of the area. The naturally occurring brackish, fresh and oil sands affected waters in the area provide a diverse range of conditions. Our assessment of the surface and subsurface flows has provided Suncor with a valuable insight to the unique aquatic habitats of the Alberta oil sands.

LABORATORY TESTS ON FISH HEALTH AND TROPHIC LEVEL EFFECTS OF WASTEWATERS FROM SUNCOR'S OIL SANDS OPERATION

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A multi-disciplinary investigation was conducted during 1995 to evaluate the health of fish which were exposed to wastewaters from Suncor's Oil Sands operation and water from the Athabasca River. This investigation was designed to [1] assess effects on major trophic components of aquatic ecosystems, assess effects on the general condition and health of fish, and [2] relate chemical characteristics to measured responses. The investigation also included an assessment of the tainting potential of various oil sands wastewaters on the flesh of rainbow trout. The investigation of fish condition and health, which was comprised of 7 and 28 d tests conducted with juvenile walleye and trout, and larval trout, included external and internal examination for pathology, analysis of livers for mixed function oxidase (MFO) activity, analyses of blood for various parameters, and measurements of growth and condition. Some of the test fish were sent for histopathological examination to allow morphological diagnosis. Challenge tests examining disease resistance and swimming stamina were also conducted on trout larval fry reared in the test waters for 28 d. The tainting potential evaluation included 200-300 mm rainbow trout exposed to one of four water types in the laboratory as well as one set of fish held within a cage in the Athabasca River upstream of the Suncor site. The flesh of exposed fish were subjected to two successive (double) triangle difference tests and two successive (double) overall preference rank tests using a taste panel. Results showed that the lowest observed effect level (LOEC) for all effects, including sensitive physiological response, was 10%. Fish exposed to lower concentrations were healthy, grew normally and responded successfully to bacterial challenge and stamina tests. No tainting was observed except in one type of wastewater.

ASSESSMENT OF (SUB)LETHAL TOXICITY OF OIL SANDS RECLAMATION WATERS USING STANDARD AND BIOCHEMICAL INDICATOR BIOASSAYS ON WATERS AND SPMD EXTRACTS

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At Syncrude's Oil Sands Plant in northeastern Alberta, large quantities of process affected waters are being produced and must be reclaimed. Both 'wet' (water capped fine tails lakes) and "dry" (composite tailings, tailings sand) landscape options are being investigated. To better understand the potential biological impacts of these processes, four waters from the experimental "wet" reclamation sites at Syncrude were tested using the standard battery of bioassays for acute and chronic toxicity, including fish (96 h rainbow trout and fathead minnow), *Daphnia* (48 h), *Ceriodaphnia* (survival and reproduction), bacteria (15 min Microtox®) and algae (*Selanastrum* growth). The most toxic water was the release water from non segregating tailings (CT) process. Some toxicity was seen in the tailings sand release water (dyke drainage water, DD). The other reclamation waters (fine tails capping water, DP, and experimental pond water, EP5) and Athabasca River water (from the settling basin, SB) were non toxic. Mixed function oxygenase (MFO) activity was highest in rainbow trout exposed for 4 d to CT water, and there was some induction measured in the DP and EP waters. Extracts of semi-permeable membrane devices (SPMDs), deployed for a 7 wk period under the ice, were tested for MFO induction in a fish cell line and analyzed for PAHs by GC/MS. Fish cell line MFO showed induction with all SPMD extracts (SB>CT>DD=DP>EP5), but the levels observed were not dramatic. The extracts of the Athabasca River water (SB) showed the highest potency. Differences in water types and possible sources of the toxicity and biochemical responses will be discussed.

WATER CAPPING OF OIL SANDS FINE TAILS: FIELD EXPERIMENTS AT SYNCRUDE'S MILDRED LAKE SITE

M.D. MacKinnon, B. Fung and T. Van Meer. Syncrude Canada Ltd., Edmonton, AB.

Large volumes of a slowly densifying aqueous suspension of fines, called mature fine tails (MFT), result from the hot water extraction process used by Syncrude at its Mildred Lake plant in northeastern Alberta to separate bitumen from oil sands. Various reclamation options based on the development of both "dry" (trafficable) and 'wet' (fluid requiring containment) landscapes are being investigated. This latter option involves retaining the MFT in geotechnically secure areas and capping it with a layer of water of sufficient depth to allow for the development of a lake ecosystem. The rheological properties of MFT and the optimization of water capping depths will minimize mixing of the layers, but the success of the approach for fine tails reclamation depends on the development of biological communities in the lake component. There will be impact from contact at the water/MFT interface and the continual input of MFT pore water into the capping layer during subsequent densification of the MFT layer. Since 1989, a series of test ponds of various sizes and compositions have been constructed to demonstrate the viability of the water-capped fine tails lakes. In this presentation, results of physical, chemical and biological studies on these various ponds will be summarized. The toxicological aspects of the water cap and the pore water release have been followed and show that there is no evidence that potentially biological detrimental components are building up in the water cap layer. A detrital layer is forming at the MFT interface and in this layer microbial communities and benthic invertebrate populations are developing. Colonization and development of diverse plankton populations in the water cap are proceeding. Fish survival and development of self-sustaining populations are being examined. The factors affecting the type and density of macrophyte growth in the littoral zones are being assessed. These test ponds provide valuable insight to the rate of development and the role that such fine tails capped lakes will play in the final reclamation and lease-closure plans. At this stage, the isolation of the fine tails in water-capped lake environments has been successful. Water capping of MFT offers a safe and environmentally acceptable option for the handling of the fluid wastes generated in oil sands operations.

SYNCRUDE'S EXPERIMENTAL WATER-CAPPED LAKE SYSTEMS - MODELLING STUDIES

R.D. Shaw[1] and M.D. MacKinnon[2]. [1]Golder Associates Ltd., Calgary, AB; [2]Syncrude Canada Ltd., Edmonton, AB.

Syncrude Canada Ltd. and Suncor Inc. operate oil sands extraction facilities in northeastern Alberta. Extraction of bitumen from the oil sands results in large volumes of fluid waste. The largest fraction is a slowly consolidating suspension (fine tails), which consists of silt and clay, water, and unrecovered bitumen. The fine tails solidify over time, releasing porewater and its associated contents. It is projected that between 500 and 1000 Mm^3 of the fine tails must be reclaimed. One option for reclamation involves disposal of fine tails under a layer of water, i.e., creation of lakes and wetlands. A number of different research programs are underway to evaluate the feasibility of this wet landscape option, including the use of experimental test pits (500 - 13,000 m^2). Data collected from these experiments are being used in computer simulation models (CE-QUAL, WASP, FGETS) to assist in our understanding of oxygen, nutrients, chlorophyll, and contaminant dynamics in the test pits and for prediction of future conditions. Modelling provides important insights into the potential for a self-sustaining ecosystem to develop in the proposed full-scale wet landscape system.

APPLICATION OF CONSTRUCTED WETLANDS FOR THE DETOXIFICATION OF OIL SANDS WASTERWATER: A RESEARCH OVERVIEW

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ABSTRACT

The dewatering of landforms created from oil sands mining activity (e.g., Dykes, Consolidated Tailing or CT areas) will inevitably result in the slow release of process-affected water such as Dyke Drainage or CT release water hundreds of years after reclamation has been completed. The potential for discharge of waters affected by Oil Sands mining operations exists during both the operation phase, when intervention is possible, and during the post abandonment reclamation phase, when natural systems must be depended on for control. This reclamation release water will likely require some level of treatment before it can be discharged into the environment, most notably, for the removal of toxicity. Research over the past five years in pilot-scale constructed wetlands has demonstrated that wetlands can offer both a treatment function. During the summer season, inorganic contaminants (e.g., ammonia, metals) and organic contaminants (e.g., hydrocarbons, naphthenic acids) can be sufficiently removed or retained in a wetlands system such that there would be no acute toxicity to trout in the discharge.

Wetlands design criteria for Dyke Drainage water have been developed using toxicological data based on the trout acute toxicity bioassay. Design criteria values ranged as follows [1] for Dyke Drainage water treated in a wetlands alone, 2,100 m^3/ha/mon, and [2] for Dyke Drainage water treated in a pond-wetlands, 6,100 m^3/ha/mon or about 32 gal/ha/min. These design criteria have a degree of uncertainty and options for winter treatment or storage need to be developed.

INTRODUCTION

Suncor Inc. Oil Sands Group (Suncor) operates a large oil sands mining and extraction operation in northeastern Alberta (Fig. 1). The extraction of bitumen from the oil sands deposits results in substantial volumes of a hydrocarbon-contaminated tailings slurry being discharged into self-contained tailings ponds for storage. In these tailings ponds, a large portion of this material forms into a fine tails layer (approx. 30 to 50% solids), consisting principally of clay and interstitial water containing organic and inorganic contaminants. The fine tails material is acutely toxic to aquatic organisms and is difficult to dewater and treat using conventional waste treatment systems.

A variety of wastewater types potentially may require treatment at the Suncor site either during the operational phase of the mine and/or after mine abandonment. Sources of these wastewaters might be Dyke Drainage or recycle water from tailings ponds; release water from the engineered treatment of tailings (Consolidated Tails); or in some other form (e.g., release water from freeze-thaw operations or from the disposal of tailings onto dry land, surface runoff from areas of consolidated tails). Whatever the source, this water will likely require treatment to remove primary toxicants, or polishing as a contingency to ensure that there are no adverse impacts on the receiving environment (e.g., the Athabasca River, nearby lakes).

The wastewater investigated in this study was Dyke Drainage (DD) water, that is, leachate from tailings ponds dykes. A suite of toxicity tests was used initially since there is no one species that can provide comprehensive information on the toxic effects of complex mixtures (Cairns, 1986). The study objective was to determine water toxicity characteristics of DD water after treatment in constructed wetlands and thereby to develop a toxicological design criterion, that is, calculating the amount of DD water that could be detoxified in a wetlands occupying a given area of land over a specified time period (i.e., m^3/ha/mon).

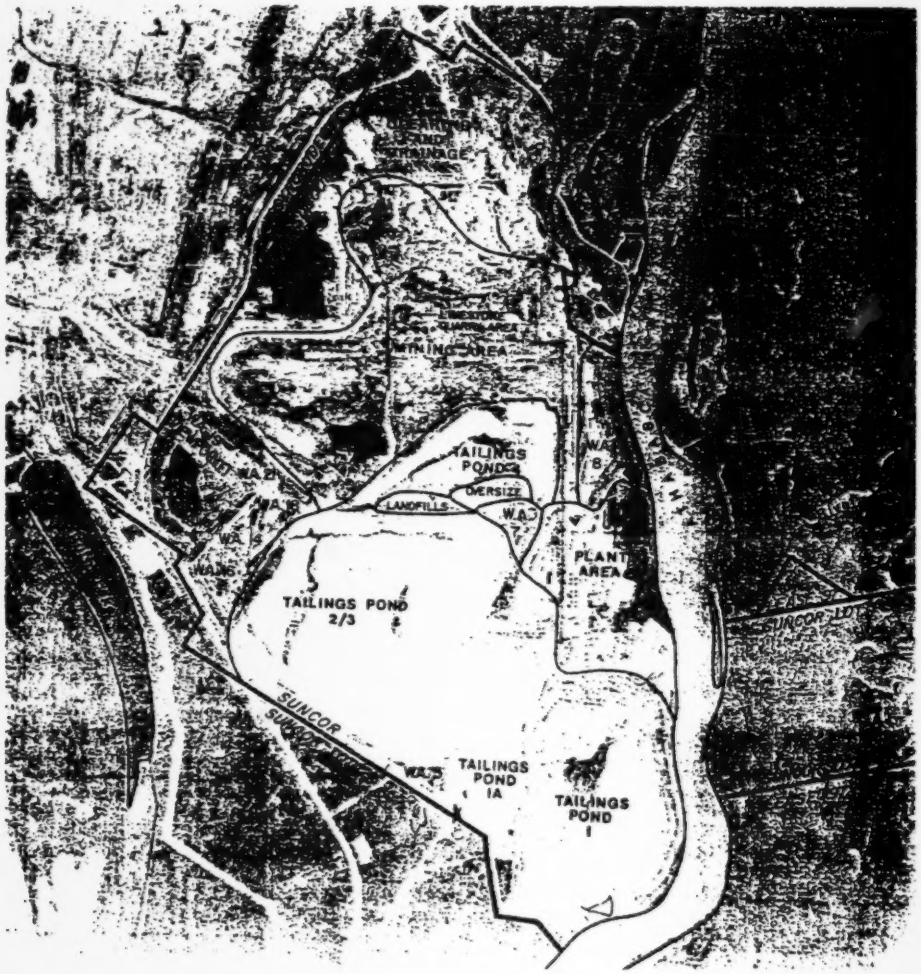


Fig. 1. Study site.

Assessment factors for treatment performance used in the overall comprehensive research study were [1] changes in concentrations of soluble and insoluble hydrocarbons (e.g., total extractable hydrocarbons, TEH) in the water column, as well as other contaminants such as naphthenic acid (NA), [2] removal rates for ammonia-ammonium, [3] *in situ* mineralization rates between treatment vs control wetlands, and [4] changes in toxicity. Conventional parameters of waste treatment efficiency (e.g., BOD, COD) were not emphasized since contributions of organic matter/detritus from wetland plants could not be reliably documented and would likely confound interpretation of these data, and since the readily biodegradable fraction of the wastewater was small (i.e., BOD <5.0 mg/L). Chemical analysis also could not satisfactorily characterize treatment performance since it is likely that only specific compounds within the TEH and NA groups are toxic and these are difficult to identify and quantify chemically. This paper focuses on reductions in toxicity using a suite of laboratory and *in situ* test organisms.

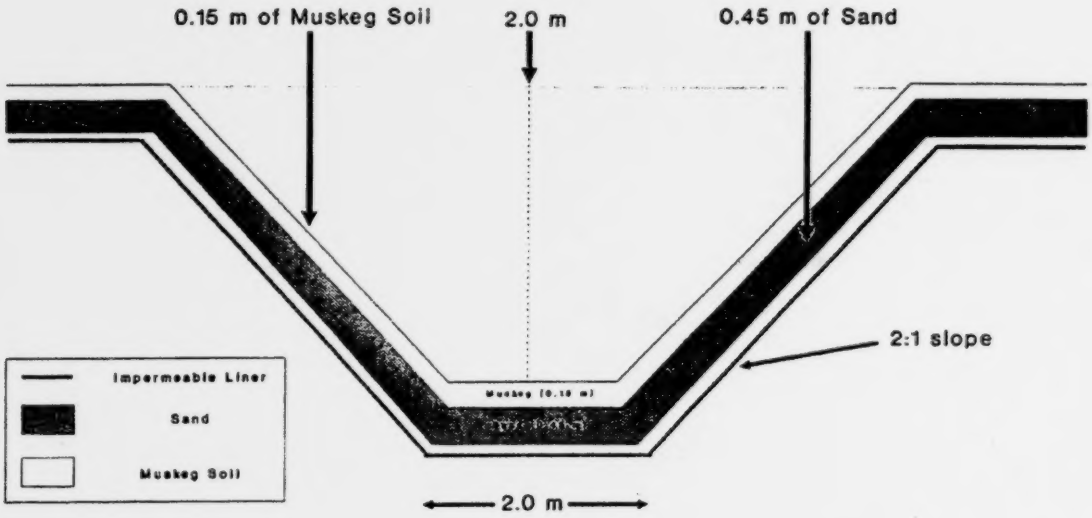


Fig. 2. Cross-section of the constructed wetlands trench (not to scale).

METHODS

Nine constructed wetlands (Figs. 2 and 3) were tested for treatment effectiveness with three types of inflow water: Pond 1A recycle water, Dyke Drainage leachate and Control (i.e., surface runoff from the south mine drainage/Loon Lake). For this paper, data from the Pond 1A wetlands has been excluded. Water input rates varied between 2 to 6 L/min for a hydraulic retention time (HRT) of between 4 and 18 d. The three replicate Control wetlands were used for comparison with the three DD water treatment wetlands. Water samples were collected from each of nine constructed wetlands trenches. Trenches T1, T4, and T8 were supplied with Control water, and T2, T6, and T9 were supplied with DD water. The constructed wetlands trenches included four sampling stations from inflows to outflows (i.e., A, B, C, D) with each station providing a data point for a specific treatment period or HRT.

Detailed methods for the various toxicological test procedures are provided in the literature as referenced. Tests utilized during four years of study included laboratory rainbow trout LC_{50} and LT_{50} tests (APHA, 1989), Microtox® bacterial test (Microtox, 1992), *Daphnia magna* 21 d life cycle test (ASTM, 1989), *Ceriodaphnia dubia* 7 d life cycle test (EPA, 1989), and *in situ* assays including the *C. dubia* 7 d life cycle test (Barjaktarovic et al., 1995), and fathead minnow *Pimephales promelas* test (Mohr et al., 1990).

RESULTS AND DISCUSSION

Laboratory *D. magna* life cycle test: In terms of sublethal toxicity using *D. magna*, treatment effectiveness was sporadic with trends of both increasing and decreasing toxicity downstream from the inflow at Station A (Fig. 4). These variable trends may be attributable to the presence of natural surfactants in the water, perhaps the result of plant or microbial metabolic processes. Most notably, DD water was only marginally toxic at the inflow (i.e., the number of progeny were only slightly less than 100% of the control) and any decrease in sublethal toxicity from inflow to outflow was marginal. Outflow water generally exhibited only slight sublethal toxicity; that is, the number of progeny ranged from 80 to 104% of laboratory controls.

There was not a strong correlation ($r^2 = 0.29$) between sublethal toxicity (i.e., percent progeny of

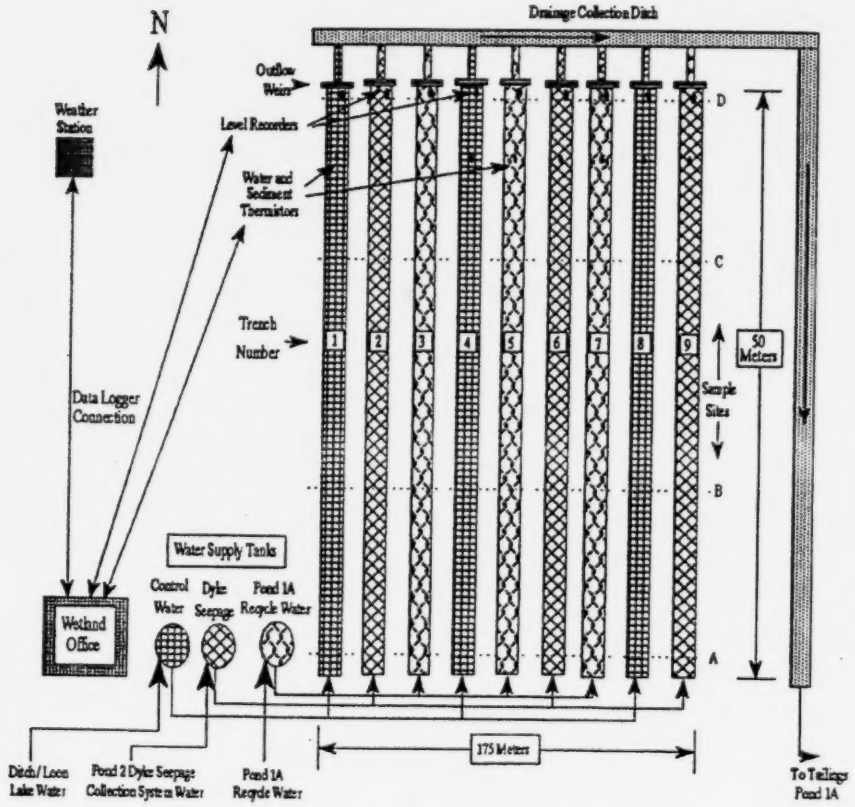


Fig. 3. A schematic map of Suncor's Constructed Wetland Research Facility.

D. magna) and TEH loading for all treatments (including the Control wetlands) and for all months (Fig. 5a). Therefore, the data were reviewed with the following exclusions [1] all Control wetlands data (since TEH values likely did not represent the same type of hydrocarbons that originate from Dyke Drainage), [2] all September data (since temperatures during this month were substantially lower compared with summer conditions and hence treatment effectiveness was also decreased), and [3] all T5 data (since the flow rates in this wetlands were altered and treatment processes may not have achieved an equilibrium during the relatively short sampling period).

Following these guidelines, data from DD treatment wetlands became more illustrative of a relationship. For example, the unclear relationship between sublethal toxicity and TEH loading rates in Fig. 5a become comprehensible as shown in Fig. 5b and having an r^2 value of 0.71 compared with 0.29 for the unselected data. Statistical treatment of these data have not been undertaken since the number of data points are limited and, more importantly, since the presence of zero values (i.e, no progeny for the *D. magna* test) would have distorted any analysis such as regressions and associated correlation coefficients.

Laboratory *C. dubia* life cycle test: As a comparison between *in situ* and laboratory methods, three samples (Control outflow, DD pond outflow, and DD wetlands outflow) were tested. Survival was 60% and 70% in the DD pond and wetlands outflow samples compared with 100% in the Control outflow, indicating a slight reduction in acute toxicity (inflow DD water was not tested). The survival LC_{50}s for all samples were >100%. The LC_{50}s for reproduction were 29 and 43% for the pond and wetlands

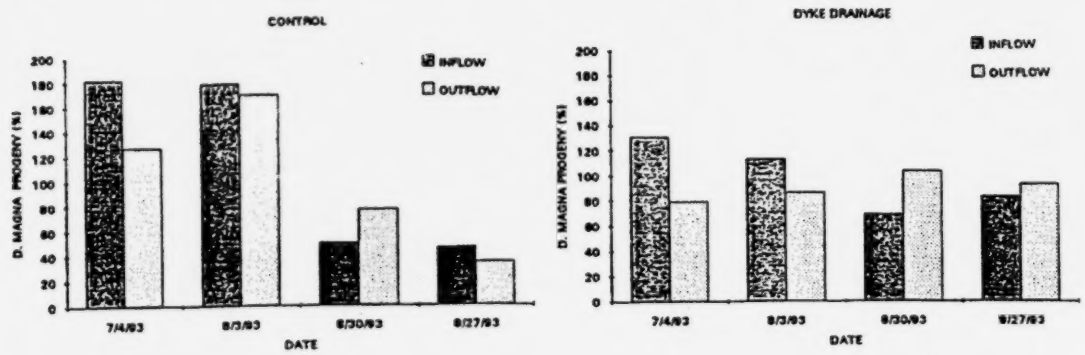
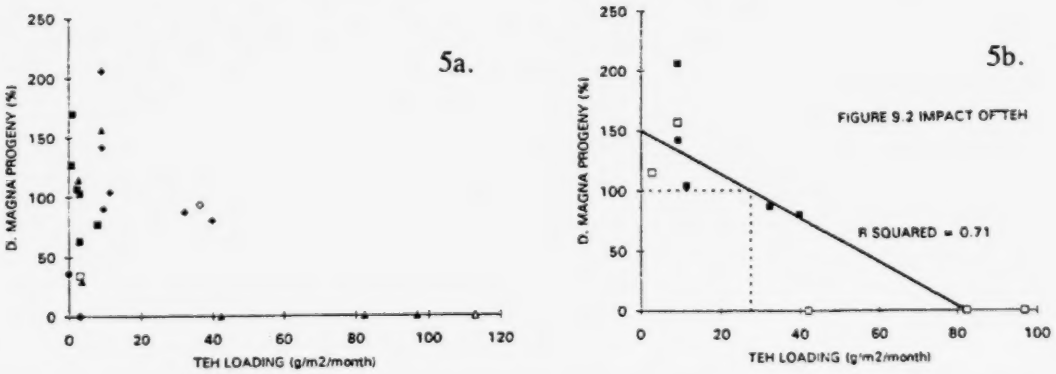


Fig. 4. Sublethal toxicity in constructed wetland inflows using the entire database for 1992 and 1993.

outflow samples and >100% for the Control outflow. These data showed substantially less acute toxicity compared with *in situ* tests, suggesting that field conditions (e.g., temperature, dissolved oxygen) likely had an adverse impact on the survival of these organisms.

In terms of chronic toxicity, the mean number of young produced per female were 0.5±1.6 and 2.9±2.6 in the DD pond and wetlands outflow samples, compared with 35.3±4.4 in the Control outflow. Therefore, laboratory data also show the presence of chronic toxicity that was not removed in the treatment system. Although the trends are similar between the *in situ* and laboratory tests, acute survival rates were different for a given sample/station. Therefore, for regulatory purposes laboratory tests should be conducted, while *in situ* tests might be used for field scoping studies.



Figs. 5 a and b. (a) The impact of TEH loading rates on sublethal toxicity in the wetland outflows using the entire database for 1992 and 1993. Symbols: ■ Control; □ Control Sept; ♦ Dyke drainage; ◊ Dyke drainage Sept; ▲ Pond 1A; △ Pond 1A Sept; ● Trench 5. (b) The impact of TEH loading rates on sublethal toxicity in wetlands outflows using selected data (i.e. omitting T5, September and Control wetlands data). Symbols: ■ Dyke drainage; □ Pond 1A. Dotted line indicates the threshold. Regression equation for the relationship is Y = 150.06 - 1.822*X.

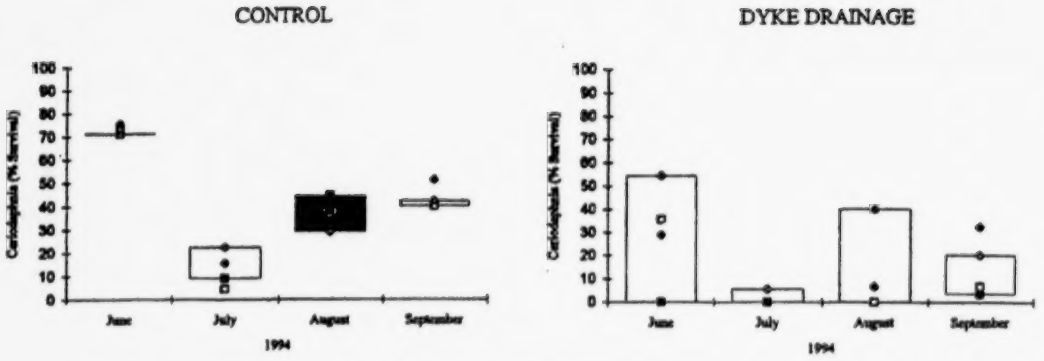


Fig. 6. Mean *Ceriodaphnia* numbers of progeny in the water column during four monthly surveys for the replicate Control and Dyke Drainage Wetlands during *in situ* bioassays in 1994. Symbols: ■ Station A; □ Station B; ♦ Station C; ◊ Station D.

In situ C. dubia tests: *C. dubia* have been used in previous in situ testing (e.g., Sasson-Brickson and Burton, 1991), and there is a wide acceptance of this species for routine laboratory tests. Unlike both Snyder-Conn (1993) and Sasson-Brickson and Burton (1991), who attempted only acute toxicity tests over 48-96 h, we elected to design a 7-10 d chronic toxicity test to observe both acute and sub-lethal endpoints which are routinely measured in laboratory experiments.

In terms of acute toxicity, there was an acutely toxic effect in the Control wetlands (Fig. 6a). Furthermore, there was no clear trend of detoxification downstream from Station A. Since percent survival was low even at the inflow, toxicity was not likely a result of some biologically or chemically produced compound from within the wetlands, but rather more likely a result of some physical or biological condition (e.g., temperature, food availability, predation). In the DD wetlands, survival rates increased substantially and consistently downstream from Station A, the mean survival rate (as a % of Control wetlands) was 73% at the outflows compared with only 2% at the inflows.

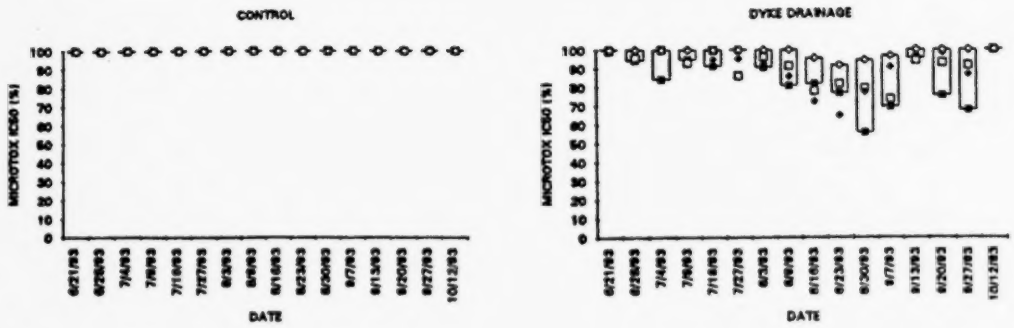


Fig. 7. Acute toxicity in constructed wetlands water downstream from inflows (Station A), June to October, 1993. Symbols: ■ Station A; □ Station B; ♦ Station C; ◊ Station D.

In terms of chronic sublethal toxicity (i.e., the total number of progeny produced), data for Control wetlands show a similar response compared with survival data, that is, the number of living progeny was relatively low at Station A (4 to 33 progeny) and decreased after the first survey in June (Fig. 6b). Chronic toxicity increased in three of the four surveys downstream from Station A, reflecting some unknown adverse impact within these Control wetlands. In the Dyke Drainage wetlands, the numbers of progeny produced were very low at all stations and there was no clear trend of detoxification. Effluent quality at Station D was poor, with a mean value of 22% (as a percent of control values).

Microtox®: Microtox® has been extensively used by Syncrude (e.g., Mackinnon and Boerger, 1986) and is considered a good screening test for toxicity in diverse environmental samples with a high degree of sensitivity to complex organic compounds (Munkittrick et al., 1991).

In terms of IC_{50} values (the concentration at which 50% of the bioluminescence is inhibited), the extent of detoxification varied a great deal during 4 mon of testing in 1993 (Fig. 7). Influent IC_{50} values (60 to 100%) were high and hence not particularly sensitive to the contaminants. In most cases, decreases in toxicity logically progressed from inflow to outflow, indicating that one or more processes were acting to remove toxicity as measured by this test. All effluent IC_{50} values were >100%. There were also no strong correlations to suggest that any parameter was associated with this toxicity (e.g., temperature, TEH).

Using IC_{20} values, there was virtually no acute toxicity in the Control wetlands at any station (Fig. 8). Therefore, the natural wetlands water appeared not to have had any adverse toxic effects as was noted for the invertebrate test organisms (i.e., increasing toxicity downstream from Stations A to D). For the DD treatment wetlands, there was a clear trend of decreasing toxicity downstream from the inflows. The mean IC_{20} for the inflows was 21%, increasing to 43% at the outflows. However, complete detoxification was never achieved (i.e., IC_{20} values were always <100%).

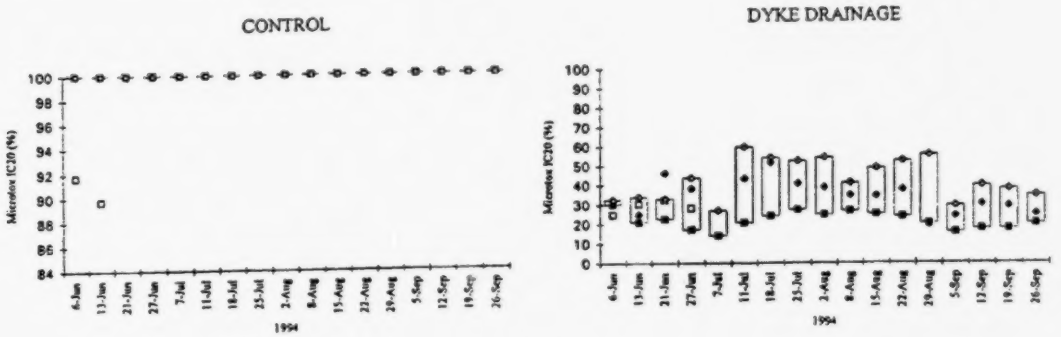


Fig. 8. Microtox® IC_{20} from Station A (inflow) to Station D (outflow) for the replicate wetlands over the 1994 study season (open bars indicate an increase from Station A to D). Symbols: ■ Station A; □ Station B; ♦ Station C; ◊ Station D.

In situ fathead minnow tests: The second organism selected for the *in situ* bioassay suite was the fathead minnow. *P. promelas* was chosen because it is native to the study area, its life cycle and sensitivity to a range of toxicants are well documented, and it is widely accepted by regulatory agencies. In the laboratory, this fish is commonly tested at the larval (24 h) or juvenile (7 to 10 d) stages in acute or chronic exposures (96 h to 7 d). To broaden the scope of endpoints analyzed through the *in situ* bioassays, a *P. promelas* embryo bioassay was used.

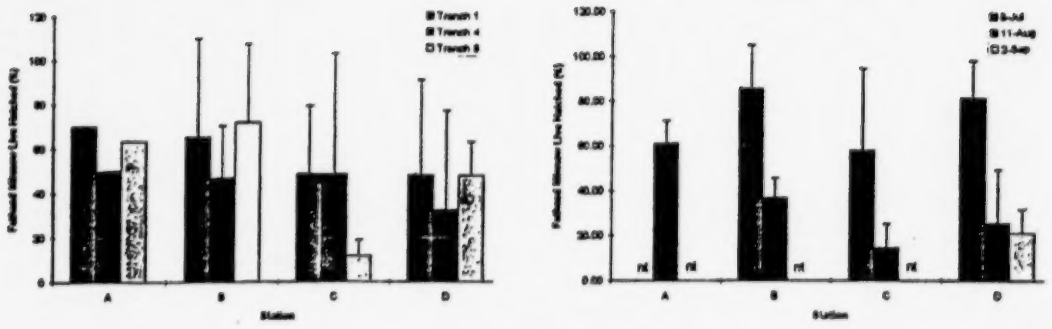


Fig. 9. Mean fathead minnow live hatched (%) in the water column at sites along the Control Wetlands by trench (a) and by date (b) during *in situ* bioassays in July, August and September, 1994.

Using the percent live hatched as the most sensitive indicator of chronic toxicity (i.e., all hatched fish that were alive at termination of the test), there was a substantial toxic impact even in Control wetlands where the percent live hatched at Station D was only 43% (Fig. 9). As with the *C. dubia* bioassays, control water became toxic to fathead minnow eggs downstream from Station A as well as over the study season. In addition, the input water tended to become toxic over time; however, these data are incomplete (i.e., limited test periods) due to a shortage of organisms. In the Dyke Drainage wetlands, the mean value at Station A was 0%, increasing to only 6% at Station D.

Laboratory rainbow trout acute toxicity test: In 1993 and 1994, complete detoxification was not achieved in the constructed wetlands treating DD water (i.e., LC50 >100%), however, a clear trend of decreasing toxicity did occur downstream from the inflow from Station A to Station D (Fig. 10). Trout LT_{50} tests were utilized as an inexpensive and practical screening test for acute fish toxicity. This test provides graduated information on acute toxicity by determining the average time for 50% of the fish to die (from 0 to 96 h). Achieving an LT_{50} of >96 h would be the equivalent of an LC50 value of >100% since both values indicate that at least 50% of the fish survived over 96 h in a 100% solution of the test water. LC_{50} represents the concentration at which 50% of the fish die and this test is often used by regulatory agencies as a criterion for acceptable effluent quality.

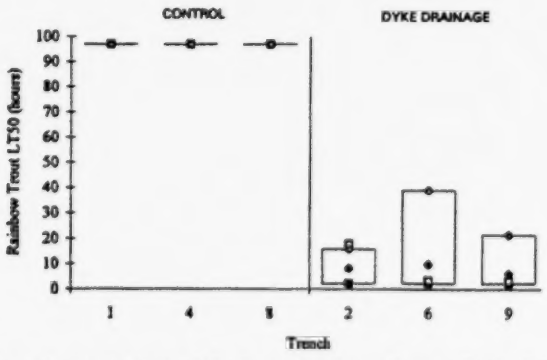


Fig. 10. Mean rainbow trout acute toxicity in water from the replicated constructed wetlands inflows (Station A) to outflows (Station D) for the entire sampling period, June to September, 1994. Symbols: ■ Station A; □ Station B; ♦ Station C; ◊ Station D.

In 1994, using trout LT_{50} data, a trend of decreasing toxicity was evident for the three replicated treatment wetlands (T2,6,9), with LT_{50} for inflows of 2 h increasing in a consistent pattern downstream to values ranging from 16 to 39 h, compared with greater than 96 h in Control trenches. Unlike many of the *in situ* bioassays, water in the Control trenches was not toxic to trout at any time over the study season. In general, the highest rate of detoxification occurred between Sites C and D, that is, toward the outflow end of each wetland where water depths were greatest and hence retention times were the longest.

In 1995, a pond was incorporated into the treatment system, upstream from the wetlands. Control wetlands water showed no acute fish toxicity (i.e., LC_{50} >100%). In contrast, DD inflow water had a mean LC_{50} of 42%, increasing to 45 to 71% at the pond outflow (the first treatment component), with further increases within the wetlands (the second treatment component) such that the final outflow water was consistently nontoxic (Fig. 11). Since 100% trout survival at the outflow of Dyke Drainage wetlands in 1994 was achieved only once, and since the retention time within the wetlands component of the 1995 system (8 d) was even less than for the 1994 system (18 d), the increased capability of the 1995 system to detoxify DD water is attributed to processes within the pond component. Although these processes (e.g., nitrification of ammonia, mineralization of organic compounds) did not result in a substantial decrease in acute fish toxicity within the pond, they evidently enhanced the detoxification process within the downstream wetlands component. In fact, ammonia-ammonium, a principal toxicant, was removed in the pond component (data not shown). This removal of ammonia in the upstream pond presumably decreased the oxygen demand of DD water entering the downstream wetlands, thereby increasing dissolved oxygen levels in the constructed wetlands such that the mineralization of some of the toxic organic compounds was enhanced (i.e., some fraction within the naphthenic acid group).

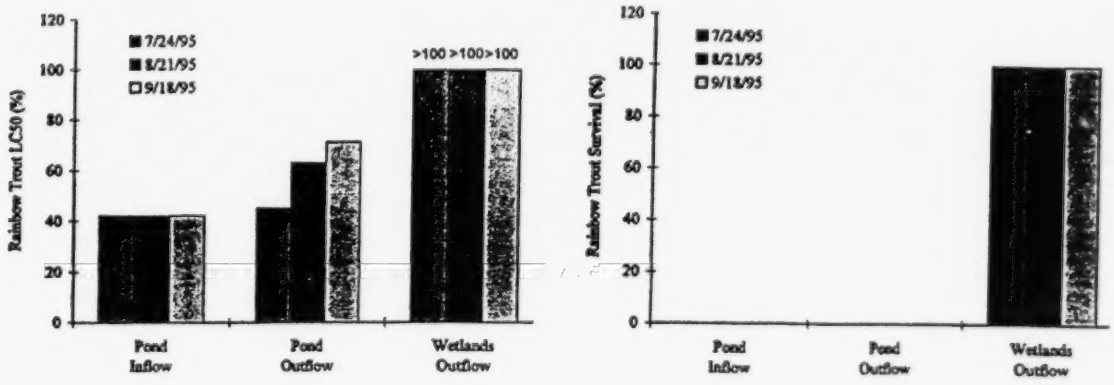


Fig. 11. Rainbow trout LC_{50}s and survival in the Dyke Drainage Constructed Wetlands, 1995.

CONCLUSIONS

Laboratory invertebrate tests were expensive ($500 to $1,100 per sample) and results showed a variable pattern of toxicity, with a sporadic pattern of increasing toxicity with increases in treatment time or HRT. Even Control wetlands water showed an indication of a toxic effect using these organisms. Nothwithstanding evidence of a chronic toxic effect produced within these constructed wetlands (including controls), the results of ecological monitoring (data not shown) suggest that a viable community of phyto- and zooplankton would inhabit these treatment wetlands. *In situ* invertebrate tests were less costly, but only if a field technician was available as a result of other tasks such as water

quality monitoring. Data from *in situ* tests also demonstrated a confounding toxic impact associated with Control wetlands water. Finally, *in situ* fathead minnow tests also showed this toxic effect in the controls.

Microtox® is a less expensive screening test (approx. $100) that did show a treatment effect in these wetlands. However, IC_{50} data were evidently not sensitive to some contaminants and not comparable with both the invertebrate and vertebrate (i.e., fish) data, that is, IC_{50} showed a non-toxic response when other test organisms showed acute toxicity. In contrast, IC_{20} data were more sensitive to contaminants in DD water, but showed only a modest response to treatment and therefore could only be used for design criteria if the data were extrapolated to a non-toxic condition (an unsound practice given the unknown and likely diverse nature of the toxic contaminants).

As a result of the above deficiencies and problems with many of the test organisms, the rainbow trout acute toxicity test was utilized as the principal toxicological parameter for development of design criteria for Dyke Drainage water in oil sands treatment wetlands since [1] it is a standard regulatory test, comprehensible to both the scientific and lay community, [2] it is relatively inexpensive (approx. $300), especially compared with laboratory invertebrate testing, [3] the data were not confounded by unknown factors in the field, such as toxic effects in Control wetlands (as was the case with in situ tests), and [4] it's level of sensitivity to the contaminants was such that it showed a non-toxic response with increasing HRTs; therefore, design criteria using this test could be formulated without any extrapolation of data.

Design criteria for the amount of water, and the area of land required, were based on a regression of toxicity removal data (using trout acute toxicity tests) versus HRT using constructed wetlands or a pond-wetlands system. Design criteria values for Dyke Drainage water were [1] in a wetlands alone, 2,100 m^3/ha/mon, and [2] in a pond-wetlands system, 6,100 m^3/ha/mon or about 32 gal/ha/min.

This latter design criterion of 32 gal/ha/min was supported by LC_{50} data for outflow water from a nearby "Natural" Wetlands located at the toe of a tailings pond dyke (hence fed mostly by DD water). The average flow rate from this wetlands was about twice the design criteria or about 60 gal/ha/min. As predicted from the above design criterion, only about one half the removal of acute toxicity of the Dyke Drainage water was achieved at its outflow (i.e., the LC_{50} at the outflow was generally about 50%). Notwithstanding this evidence, these design criteria have a degree of uncertainty due to the natural variability of many environmental processes which might have contributed to the detoxification of this water. In addition, the benefits of enhancement features not discussed in this paper (e.g., phosphate addition, biological filtration) have only been explored in preliminary experiments and need to be confirmed.

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ECOLOGICAL VIABILITY OF WETLANDS RECEIVING OIL SANDS EFFLUENT

L.I. Bendell-Young, A.P. Farrell, C.J. Kennedy, A. Kermode, M.M. Moore and A.L. Plant. Simon Fraser University, Department of Biological Sciences, Burnaby, BC.

A suite of ecological relevant endpoints were compared among constructed wetlands receiving either unamended or amended (addition of phosphate to enhance microbial degradation of organics or addition of gypsum) dyke seepage waters versus a control constructed wetland receiving lake water and a reference wetland located off the Suncor lease. Endpoints included [1] benthic macroinvertebrate community structure, chironomid density and biomass, and the incidence of chironomid mentum deformities, [2] plant growth and photosynthetic ability, [3] fish stress response as measured by %haematocrit (%Hct), %leucocrit (%Lct) and gill histology, and [4] the mutagenic potential of sediment dwelling chironomids. The wetland receiving unamended dyke seepage water was unique from 11 other wetlands as indicated by [1] a distinct benthic community structure including a high biomass of chironomids, [2] a significantly lower maximum growth plus elevated photosynthetic rate in cattails grown in water and sediments from the wetland, and [3] increased %Hct, depressed %Lct, and ultimately death in fish held beyond 14 d in the unamended seepage versus those held in reference and control wetlands. Of the various constructed wetlands, wetlands receiving dyke seepage waters amended with phosphate and gypsum (Consolidated Tails (CT) water) contained a benthic community comparable with the offsite reference wetland. However, CT water and CT water plus phosphate compromised fish health as indicated by elevated %Hct and depressed %Lct. Fish held in the wetland receiving dyke seepage water amended with phosphate appeared to be the least stressed compared to all other treatment waters. Mutagenic potential of invertebrates and sediments from the wetland receiving unamended dyke seepage was not different from that of the control wetland. Using the above ecologically relevant endpoints to define ecological viability (i.e, a healthy system), the wetlands receiving unamended dyke seepage water appears to be ecologically 'marginal.' The impact of this wetland on higher trophic levels (i.e., waterfowl) needs to be addressed.

PHYTOTOXICITY OF RECLAIMED FINE TAILS AND TAILINGS SANDS

S. Renault and J.J. Zwiazek. University of Alberta, Department of Renewable Resources, Edmonton, AB.

Oil sand operations produce large amounts of wastes that contain toxic components. Greenhouse experiments were conducted to determine the phytotoxicity of soil enriched fine tails and consolidated tails in raspberry, white spruce and jack pine seedlings. All of the white spruce seedlings survived in the presence of 30% consolidated tails and 30% fine tails, while the survival rate for jack pine seedlings in the same conditions was 40%. Eighty five percent of the raspberry seedlings survived in the presence of 30% consolidated tails while only 40% survived in the presence of 30% fine tails. In jack

pine, shoot and root growth were not altered in the presence of consolidated tails and fine tails, while the shoots of white spruce seedlings showed reduced lateral branching. In raspberry, both root and shoot growth were reduced by treatment with 30% consolidated tails and fine tails. The 30% concentration of consolidated tails did not affect photosynthesis, transpiration and respiration rates and did not alter membrane integrity in the studied species. An identical concentration of fine tails damaged the cell membranes in raspberry seedlings and affected their respiration and transpiration rates. A higher, 50%, concentration of consolidated tails and fine tails had a negative impact on all of the examined parameters in the studied plants.

MINING
SESSION CHAIR: M. RANKIN

PROTECTIVE MECHANISMS OF DISSOLVED ORGANIC CARBON (DOC) AGAINST THE PHYSIOLOGICAL EFFECTS OF COPPER AND CADMIUM ON TROUT

J. Richards[1], L. Hollis[2] and R. Playle[2]. [1]University of Waterloo, Department of Biology, Waterloo, ON; [2] Wilfrid Laurier University, Department of Biology, Waterloo, ON.

Rainbow trout (*Oncorhynchus mykiss*, ~200g) were exposed to a mixed metal solution of 0.15 mM Cd and 0.75 mM Cu and ~40 mg/C×L commercial humic acid to determine if DOC is protective against metal induced physiological changes. Trout were fitted with dorsal aortic cannulae which allowed repetitive blood sampling for determination of ionoregulatory and respiratory status of the fish during a 5 d exposure. Humic acid prevented Cd and Cu accumulation in fish plasma and provided a clear protective effect against Cd and Cu induced blood gas and acid base disturbances. There were no deleterious effects of 40 mg/C×L humic acid on blood gas or plasma ion status. Natural DOC isolated from a marsh was also examined at an environmentally realistic concentration (~5 mg/C×L) to determine if the same protective effects occurred. Natural DOC also completely protected against Cd and Cu accumulation in fish plasma and protected against blood gas disturbances over the 5 d metal exposure. Dissolved organic carbon is clearly an important modifier of metal toxicity to fish, and it has no adverse physiological effects itself.

PREDICTIVE ASSESSMENT OF ECOLOGICAL RISKS IN URANIUM MINING

D.R. Hart, P. McKee and F. Garisto. Beak Consultants Ltd., Brampton, ON.

Environmental assessments at uranium mines frequently involve forecasting of dose and risk to ecological receptors. Potential effects of habitat disturbance and increased site access should also be considered. Current modelling and risk assessment approaches are illustrated using case studies related to mine opening and decommissioning. These cases illustrate the importance of capturing uncertainties with respect to radionuclide release, physical dispersion and food chain transport, and of using site-specific parameter measurements to reduce uncertainty. Radiation dosimetric models for biota are compared and uncertainties related to plant and animal dosimetry are discussed. The importance of integrating predictive modelling with environmental effects monitoring programs is emphasized.

METALLOTHIONEIN IN FISH: APPLICABILITY TO METAL MINING BIOMONITORING PROGRAMS AND RESEARCH NEEDS

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INTRODUCTION

In 1994, the Aquatic Effects Technology Evaluation program (known as "AETE") was initiated as a joint venture between the mining industry through the Mining Association of Canada, several federal government departments, and representatives from some of the provincial governments. The AETE Program is administered and managed through the Canada Centre for Mineral and Energy Technology (CANMET) with Natural Resources Canada. The main objectives of the program (CANMET, 1994) are two-fold [1] to assist the Canadian mining industry to meet its environmental effects monitoring and related requirements in as cost-effective manner as possible, and [2] to benefit the Canadian environment by evaluating new and existing monitoring technologies for the assessment of environmental impacts with indications of the benefits and weaknesses of each technology.

The Program deliverables will be a series of reports on appropriate, cost-effective methods of determining the biological and non-biological impacts of mine effluents on Canada's lakes, rivers and streams. The three main elements of the Program are [1] acute and chronic toxicity testing, [2] water and sediment monitoring, and [3] biological monitoring. The technologies to be evaluated within each of these elements are defined by Technical Committees comprised of representatives from universities, industry and government.

As indicated in the title, the mandate of the Biological Monitoring Technical Committee is to evaluate biological methods of assessing the impacts of mine effluents on aquatic biota. The taxonomic groups under evaluation include fish, benthic invertebrates, zooplankton and phytoplankton. Fish are assessed at community, population, organism, tissue, and molecular levels. Initial meetings by members of the Fish Group discussed many parameters, as shown in Table 1, that have been shown to be metal sensitive or to be related to the growth and reproduction of fish. Further discussions and integration with the other major elements of the Program resulted in a considerable reduction in the list of parameters (Table 2) for full field evaluation. Out of approximately seventy molecular parameters considered (Table 1), metallothionein was selected as the only biochemical response to be evaluated (Table 2) along with parameters at other levels of biological organization.

Metallothionein (MT) is a low-molecular weight, cysteine-rich protein that is found in the tissues of aquatic invertebrate and vertebrate animals (Roesijadi, 1992). MT binds Cd, Cu, Hg, Ag and Zn. The functions of the protein are thought to include the regulation of the essential metals, Cu and Zn, and the de-toxification of excess amounts of those metals and of non-essential metals, such as Cd and Hg. MT is promoted for use in bio-monitoring programs as an indicator of the biological availability of these metals, and as an early response related to the development of resistance to the toxicity produced by these metals (Roesijadi, 1992). There are a considerable number of laboratory studies and a limited number of field studies that demonstrate increased resistance to metal toxicity in fish with elevated concentrations of metallothionein in their tissues. In other words, there is evidence of a relationship between this response at the molecular level to the health of individual animals.

METHODS

In our laboratory, MT is analyzed by using inorganic Hg to displace *in situ* bound Cu, Cd and/or Zn (Dutton et al., 1993; Klaverkamp and Wautier, 1996). MT is not an enzyme and can be analyzed under conditions that would be highly unfavourable to the more labile enzymatic proteins. Inorganic Hg is

added and binds to MT while proteins other than MT are removed by the use of heat and 20% trichloroacetic acid. This Hg displaces the other metals and occupies the sites that were binding the other metals. MT concentrations are estimated by the use of an analytical standard, MT from rabbit liver available from SIGMA (Sigma-Aldrich Canada Ltd.), and on the basis of 7 gram atoms of Hg being bound to one MT protein molecule. It is important to emphasize that the stability of MT under these heat and acidic conditions allows the protein to remain in solution, and is a basis for its analyses. In our laboratory at the Freshwater Institute, the cost per analysis is approximately ten dollars, which covers the technicians salary and the analytical materials and supplies. Approximately 60 analyses can be conducted in a week.

Table 1. Measurements on fish considered by the Biological Monitoring Group of the "Aquatic Effects Technology Evaluation" (AETE) Program.

COMMUNITY:	Electrofishing community survey of rivers.
POPULATION:	Size. Age structure.Recruitment. Mortality rate.
INDIVIDUALS:	Size. Age. Growth rate. Condition Factor. Gross anomalies. Parasites. Fecundity.
ORGANS:	Size of liver, kidney, gonad and spleen. Histopathology of liver, kidney, gonad, thyroid and gill. Skeletal bone: Strength, composition and elasticity.
MOLECULAR:	Metallothionein. MFO induction. Free radical damage. Cytochrome oxidase (CN). Amino-Levulinic Acid Dehydratase (Pb). Succinic dehydrogenase and other mitochondrial enzymes (As). Xanthine oxidase. Superoxide dismutase. Allantoinase. Oxidative stress. Lysosomal degradation. Lipase. Sorbitol dehydrogenase. Glutamate dehydrogenase. Lactate dehydrogenase. Glutamate. Pyruvate transaminase. Glutamate. Oxaloacetic transaminase. Amylase. Aspartate. Aminotransferase. Alanine. Aminotransferase. Leucine. Aminonaphthlamidase. Proximate analyses. RNA/DNA ratios. O_2:N_2 ratios. mRNA expression. Adenylate Energy Charge. Glycogen. Lipid. FFA. Triglycerides. Triacylglycerol. Cholesterol. Glucose. Protein. Steroid hormones. Vitellogenin. Gonadotropic hormone. ATPase. Phosphorus. Calcium. Porphyria. Electrophoretic protein separations. Chromosomal aberrations. Sister chromatid exchange. White blood cell ratios. Lactate. ACTH. ChEI. Cortisol. Corticosteroids. Neuroamines. Monoamine oxidase. Carbonic anhydrase. Alkaline/Acid Phosphatase. Lipofuschin. Creatinine. Bilirubin. beta-Glucuronidase.
CHEMISTRY:	Metal concentrations in critical target organs.

MT analyses were conducted on tissues dissected from live, anaesthetized fish. The individual tissues were immediately placed in Whirlpac bags, frozen on dry ice and transported to the Freshwater Institute. Tissues were kept at -90°C until analyzed.

Metals in sediments from Yellowknife Bay of Great Slave Lake were analyzed by CanTest Ltd. (Vancouver, BC) according to methodology described in Jackson et al. (1996). Statistical analysis of metals in sediments was performed by one-way analysis of variance with $\alpha = 0.05$. Significant elevations in mean values at effected sites, relative to the reference site, were determined by Dunnett's one-tailed t-test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

MT results are presented for fish from two very different freshwater habitats. One of these involved the analyses of fish from Great Slave Lake, the fifth largest lake in North America, in the Northwest

Territories (NT), and the other from a small lake in the Experimental Lakes Area of northwestern Ontario. These two studies were conducted independently and did not receive funding from the AETE Program.

Table 2. Measurements on fish for actual evaluation during the first field season under the AETE Biological Monitoring program.

COMMUNITY:	Electrofishing in rivers.
POPULATION:	C.P.U.E. (provides the Individuals).
INDIVIDUALS:	Size. Age. Growth rate. Condition Factor. Gross anomalies. Fecundity.
ORGANS:	Size of liver and gonads. Histopathology of liver, kidney, gonad and gill.
MOLECULAR:	Metallothionein.
CHEMISTRY:	Metal concentrations in liver, kidney, gill and muscle.

The first of these studies involves the analyses of lake whitefish and northern pike from Yellowknife Bay of Great Slave Lake. One of the objectives of the study was to evaluate whether these fish were responding to elevated concentrations of Cd, Cu, Hg, and Zn found in the sediments near the discharge points for treated effluents released by the two gold mines in the area (Fig. 1). Fish and sediments were collected at three sites in Yellowknife Bay. Site 1 served as a reference point and was approximately 7 km from Site 2, which was near the discharge point for the Miramar-Con mine, and about 12 km from Site 3, which was near the discharge point for the Royal Oak-Giant mine. Treated effluent from the Miramar-Con mine drains through a series of 4 small lakes before reaching Yellowknife Bay. Treated effluent from the Royal Oak-Giant mine drains into Baker Creek which discharges directly into the bay. Metal concentrations in surficial sediments from these sites are presented in Table 3. In comparing the most contaminated site, Site 3, to the reference site, Site 1, there are 3.6-, 35-, 26-, and 10-fold higher concentrations of Cd, Hg, Cu and Zn, respectively in the sediments at Site 3.

Table 3. Concentrations, expressed as mean±S.E.M., of metals in surficial sediments of Yellowknife Bay, Great Slave Lake, NT.

Metal	Site Number		
	1*	2**	3***
Cd (mg/kg, dry wt.)	0.20±0.04	0.53±0.05	0.72±0.04
Cu (mg/kg, dry wt.)	16.2±1.5	46.6±5.9	423±50
Zn (mg/kg, dry wt.)	31±6	88±9	301±16
Hg (mg/kg, dry wt.)	3.5±1	22±2	122±7

*Reference site, north side of Horseshoe Island.
**Site near discharge point for Miramar - Con mine.
***Site near discharge point for Royal Oak - Giant mine.

Fig. 1. Study site.

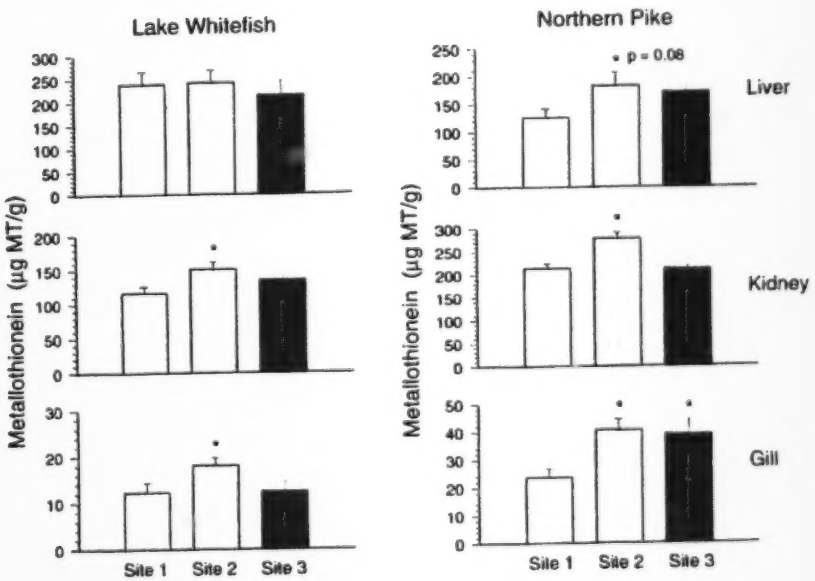

Fig. 2. Yellow Bay, *Means significantly greater than reference Site 1 ($p < 0.05$); Dunnett's one-tailed t-test.

Fig. 2 presents the MT data for liver, kidney and gill of twenty lake whitefish (left panel) and twenty northern pike (right panel) collected from each of these sites. Although these data do not provide clear dose-response relationships to the degree of contamination observed in the sediments, they do demonstrate some degree of elevated MT concentrations in fish from the contaminated sites. Lake whitefish from site 2 had significantly higher MT concentrations in their kidneys and gills, relative to reference site 1. Northern pike from site 2 had higher MT concentrations in all three tissues, and pike from site 3 had higher MT concentrations in gill and had marginally higher ($p = 0.08$) concentrations in liver. Because many factors control the biological availability of metals in sediments (Mason and Dragun, 1996), numerous studies have shown poor relationships between total metal concentrations in sediments and bioavailability (Luoma, 1989; Ankley et al., 1994; Krantzberg, 1994).

Evaluating the MT data for fish from site 1 using a statistical power analyses with the likelihood of a Type I Error (α) set at 0.05, the power of the test ($1 - \beta$) at 0.95, and delta (δ = the magnitude of change) at 100%, we found that 6, 9 or 17 lake whitefish would be required to detect a doubling of MT in kidney, liver and gill, respectively. For northern pike, these analyses demonstrated that 3, 10, and 11 pike would be required to detect a doubling of MT in kidney, liver and gill, respectively.

The second study involved small oligotrophic lakes in the Experimental Lakes Area, located in northwestern Ontario. One of these lakes, Lake 382, has a surface area of 37 hectares and a maximum depth of 13 metres, and it received experimental additions of Cd during the open-water seasons from 1987 to 1992 (Lawrence et al., 1996). Rationale for selecting Cd, experimental highlights and factors involved in the premature termination of the experiment are discussed in Malley (1996). Lake trout and white suckers were sampled from this lake and from a similar lake in the area which served as a reference during this period and for two years after Cd additions ceased. Table 4 presents information on the amounts of Cd added, the time period of additions, and the concentrations in ng/L of Cd in the epilimnion. These Cd concentrations are expressed both during the additions period only and as the overall annual average. The highest concentration achieved during the period of addition was 185 ng Cd/L, which is below the Water Quality Guideline of 200 ng Cd/L for the protection of freshwater life in soft water systems. The overall annual averages for Cd concentrations were well below this Guideline.

Table 4. Cadmium additions to Lake 382, Experimental Lakes Area.

Year	Cd addition Added (g)	Add'n Period*	[Cd](ng/L) (add'n per)**	[Cd](ng/L) (ann aver)***
1987	978	23/vi - 29/x	75	38
1988	641	5/vi - 11/x	60	55
1989	780	6/vi - 31/x	100	60
1990	1442	5/vi - 27/x	126	50
1991	1546	22/v - 27/x	178	67
1992	1289	15/vi - 27/x	185	78
1993	0	n/a	n/a	64
1994	0	n/a	n/a	21
Total:	6676			

*The actual period of Cd additions; i.e 23/vi - 29/x is from June 23rd to October 29th.
**Mean epilimnetic Cd concentration (in ng/L) during the actual period of Cd additions.
***Mean epilimnetic Cd concentration (in ng/L) over the entire year.
n/a No Cd additions.

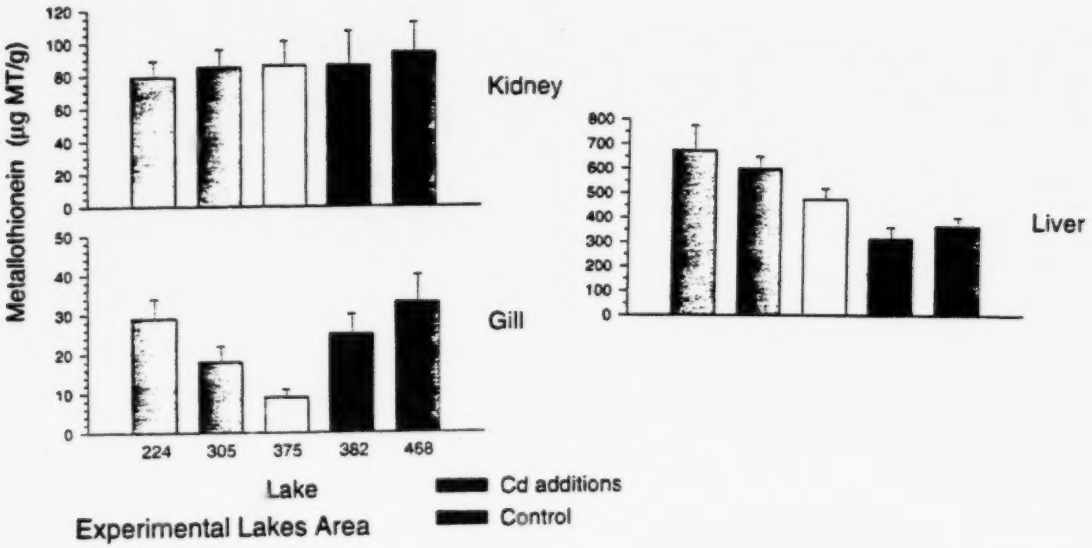

Fig. 3. White sucker, reference data.

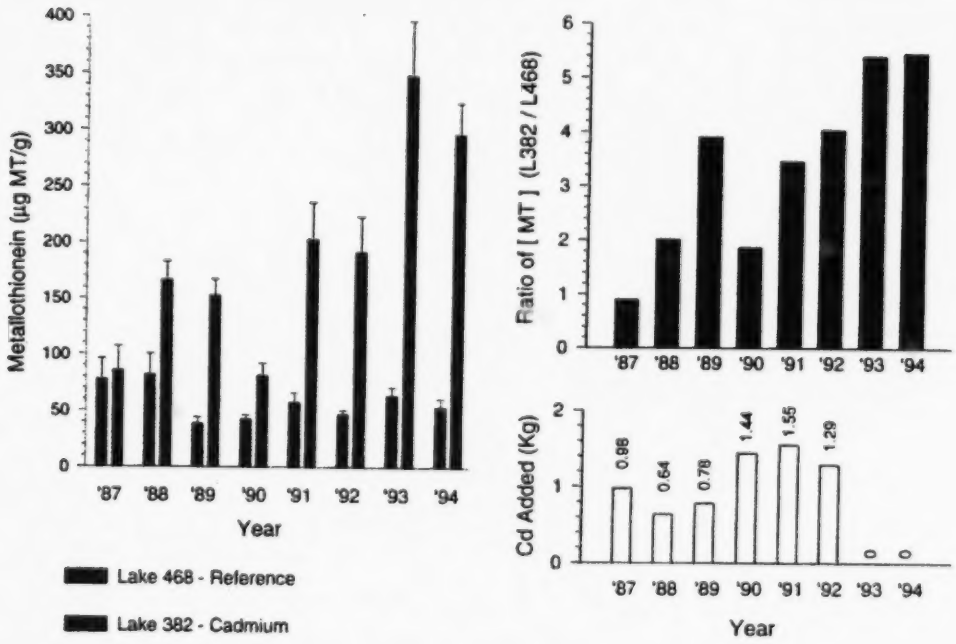

Fig. 4. White sucker kidney.

Before Cd additions began, we obtained fish from several lakes in the Area in order to assess lake-to-lake variability in tissue concentrations of MT (Fig. 3). The data demonstrate that MT concentrations in white sucker liver can vary by a factor of approximately 2.2. The fish from Lake 382 had the lowest pre-addition concentration of liver MT, whereas the lake, Lake 468, chosen as a control over the entire duration of the experiment had the second lowest. Concentrations of MT in gill were the most variable with differences of approximately 3.7 fold. Concentrations of MT in kidney were the most consistent in fish from these five lakes. In subjecting the data for fish from Lake 468 to the statistical power analyses as described above, we found that 5, 12 or 14 white suckers would be required to detect a doubling of MT in liver, kidney and gill, respectively.

Fig. 4 (left panel) presents MT data for kidney of white sucker from the Cd-treated lake and the reference lake for the period of cadmium-additions and two years after additions were stopped. Although there were year-to-year differences, fish from the Cd-treated lake consistently had higher MT concentrations, with the exception of the first year when there were no differences in fish from the two lakes. When these concentrations are expressed as ratio, [MT] L382/L468 (Fig. 4 right panel), we see that this ratio goes from 0.9 at the start of the experiment to a high of 5.5 two years after Cd additions ceased.

In the later two years of the experiment only, we captured and analyzed visceral contents of a small forage species, pearl dace. In this case, the reference lake was Lake 114. Concentrations of MT were over 4-fold higher in the viscera of fish from the cadmium-treated lake. It is unfortunate that only minimal effort was devoted to small forage species, because they may have advantages as a sentinel species.

In conclusion, some recommendations, expressed as questions, for additional research on the use of metallothionein in fish as a bio-monitoring tool are presented in Table 5. Like all other tools and approaches used in biomonitoring, the use of MT in fish can benefit from additional research. These recommendations are restricted to biological and toxicological issues for fish, and they do not address other essential issues, such as the need for QA/QC in MT analyses, the utility of MT in aquatic invertebrates and benthic organisms, and the need to relate responses to metal loadings.

Table 5. Some research requirements for the use of metallothionein in fish in Bio-Monitoring Programs.

1. Relevance to "health" of individuals and populations?
   a. "Biological costs" of acclimation to metal toxicity?
   b. What is "Critical Target Organ"? Liver? Kidney? Gill? Others?
   c. Are each of these questions, i.e. 1a. and 1b., metal-specific and/or fish species-specific?

2. Fish species differences?
   a. Sediment-feeders vs plankton-feeders vs top-predators?
   b. Small fish? Less migratory? Forage species? Young-of-the-year for large species? What is/are good sentinel species?

3. Spatial and temporal differences?
   a. Spatial: influence of geology on "baseline" MT; i.e. highly mineralized vs less mineralized areas?
   b. Temporal: seasonality? Sex differences? Influence of spawning?

Requirement 3 (spatial and temporal differences) consists of the basic need to know "natural variability" and to establish a data base on MT concentrations in fish. To that end we have had the opportunity

to take some steps in building a data base and have MT data for [1] white suckers and northern pike from northern Alberta, Saskatchewan, Manitoba, Ontario and eastern Quebec, [2] lake trout from Lake Ontario and Lake Superior, [3] burbot, lake whitefish, walleyes, and perch from Alberta, Saskatchewan, Ontario and the Northwest Territories, and [4] Atlantic salmon from rivers in New Brunswick.

A thorough review of the literature pertaining to the use of MT in biomonitoring programs for the mining industry of Canada is being conducted as part of the AETE program (Project # 2.2.1). This review (Couillard, 1996) provides additional examples on the use of MT in natural populations of fish and aquatic invertebrates, and gives recommendations on the need to understand the fundamental mechanisms of and acclimation to metal toxicity.

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LABORATORY SCREENING OF SUBLETHAL TOXICITY TESTS USING EFFLUENTS FROM CANADIAN METAL MINING SITES

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The Aquatic Effects Technology Evaluation (AETE) program was established to review technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of

provincial governments and, is coordinated by the Canadian Centre for Mineral and Energy Technology (CANMET). The objective of the chronic toxicity sub-program is to evaluate sublethal toxicity tests for estimating potential sublethal impacts resulting from the discharge of metal mining effluents in as cost-effective manner as possible. In the first project, eight mining effluents were used to evaluate the performance of eight sublethal 'chronic' bioassays. The bioassays compared are *Ceriodaphnia dubia* survival and reproduction, fathead minnow survival and growth inhibition, *Selenastrum capricornutum* microplate growth inhibition, Microtox® chronic, nematode survival and reproduction, rainbow trout early life-stage survival (7 d test), *Lemna minor* growth inhibition and multi-species microplate algal growth inhibition. Receiving waters were used as dilution waters for bioassays with fathead minnow, *Ceriodaphnia*, rainbow trout embryo and *S. capricornutum*. Most (88%) of the bioassays were judged as valid tests according to the protocols despite the fact that the organisms were not acclimated to the receiving waters prior to the tests. Successful tests included the *S. capricornutum*, *L. minor*, multispecies phytoplankton, *Ceriodaphnia*, fathead minnow and the Microtox® chronic tests. The IC_{25}s indicate that the most sensitive were the algal growth inhibition assays (the *S. capricornutum* and the microplate multispecies tests), followed by the *L. minor* and *Ceriodaphnia* assays. The least sensitive were the Microtox® chronic and fathead minnow growth/survival tests. Effluent exposure resulted in stimulation in certain cases, especially in the Microtox chronic test.

BIOPHYSICAL EFFECTS OF URANIUM MINING IN CANADA AND SOME SUGGESTIONS FOR BIOMARKERS OF EFFECTS

S.M. Swanson and N. Roest. Golder Associates Ltd., Calgary, AB.

A review of the biophysical effects of uranium mining and milling in Canada showed that impacts have decreased with time. Mines that operated in the 1950's and 1960's caused degradation in water quality and elevated radionuclide levels in plants and animals exposed to mining and milling wastes. Effects on biota included reduction or elimination of sensitive groups because of acidification, high ammonia, siltation or increases in salinity. The impacts from the early mines have been or are being actively mitigated as a part of decommissioning and reclamation programs. The impacts of mines operating from the mid 1970's to the present are much less pronounced than those observed at the older mines. Effects on water quality are usually confined to the first one or two receiving lakes or streams. Radionuclides (usually U), metals (Ni, Mo), As and salinity are the parameters that are most commonly elevated above baseline conditions. Elevated contaminants in sediments are also found in the first receiving water bodies as well as in areas that have been extensively explored. Radionuclides in biota are usually at baseline levels, with the exception of U in macrophytes and some instances of U and ^{210}Pb in fish. Acutely toxic effects and sublethal effects on biota have sometimes been observed in laboratory tests on effluents or mine water from the newer mines, largely in relation to Ni toxicity. Longer term effects on biota appear to be primarily related to increased salinity, however, Ni and As toxicity are also implicated at some sites. Indirect effects on fish through habitat changes may also have occurred. There are several candidates for sensitive indicator species at U mine sites. These species would belong to the following groups [1] members of the genus *Pinus*, [2] mice and voles, [3] early life stages of fish, [4] amphibians, and [5] certain species of algae. These groups contain species that are sensitive to both radiation and metals. There is a wide choice of measurements to use as indicators of effects. Useful indicators of radiation and/or metal effects at the individual organism level includes [1] chromosome aberrations, [2] fertility/fecundity, [3] haematological parameters, and [4] growth form or growth rate. Useful indicators of effects at the population or community level includes [1] species richness, [2] species composition, [3] recruitment, [4] survivorship, [5] age and size distributions, [6] disease incidence, and [7] behaviour.

ARCTIC CONTAMINATION
SESSION CHAIR: M. ALAEE

METHYL SULFONE PCB AND 4,4'-DDE METABOLITES IN THE POLAR BEAR FOOD CHAIN: THE RELATIVE IMPORTANCE OF BIOACCUMULATION, FORMATION AND RETENTION

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Chlorinated hydrocarbon contaminants bioaccumulate and biomagnify in the Arctic marine ecosystem. No studies have been reported that demonstrate that methyl sulfone- ($MeSO_2$-) PCB or 4,4'-DDE metabolites bioaccumulate within any food web, including the polar bear food chain. For this reason, whole arctic cod, ringed seal blubber and polar bear fat are analysed for $MeSO_2$-PCBs and -4,4'-DDEs and their precursor PCB and 4,4'-DDE compounds. Approximately 10% of the metabolism of PCBs not substituted at one of the *meta-para* positions proceeds by mercapturic acid metabolic pathway (MAP) in arctic ringed seal to form bioaccumulating $MeSO_2$-PCBs, indicating that metabolic pathways other than the MAP were usually favoured. Complete metabolism of these PCB congeners occurs in polar bear (<0.05 µg/kg, lipid weight), however, a similarly small proportion forms $MeSO_2$-PCBs. $MeSO_2$-PCBs or -4,4'-DDE were not detected in arctic cod (<0.05 µg/kg, lipid weight), therefore all of the metabolites in seal must have been formed by the seal. $MeSO_2$-CB132 is bioaccumulated entirely from seal by polar bears. A number of other congeners also appear to be bioaccumulated mainly from seal to bear. The formation and/or slow clearance of $MeSO_2$-CB70, -CB91, -CB149 and -CB141 occurs in the bear. Only a small proportion of 4,4'-DDE metabolism favoured $MeSO_2$-metabolite formation in seal (ca. 3%) and bear (ca. 1%). In conclusion, $MeSO_2$-PCB uptake from seal is an important source of $MeSO_2$-PCBs in bear, although selective bioaccumulation, formation and retention of $MeSO_2$-PCBs all influence the resulting $MeSO_2$-PCB congener levels and patterns in both seal and bear.

MFO INDUCERS FROM REFINERY EFFLUENT, MACKENZIE RIVER, NORMAN WELLS

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Semipermeable membrane devices (SPMDs) were deployed for 11 to 12 d in Mackenzie River waters upstream and downstream of, as well as in the skimmer ponds and effluent stream of, Imperial Oil, Norman Wells. Extracts of SPMDs were dosed to fish liver cells (PLHC-1, *Poeciliopsis lucida* hepatocarcinoma cells, a liver tumour cell line from a top minnow) and mixed function oxygenase (MFO) was determined by measuring the activity of ethoxyresorufin-*O*-deethylase (EROD) in the cells. SPMDs deployed at the upstream (7 km) site had no more MFO inducers than present in trip blanks. SPMDs from the effluent contained potent MFO inducers, with potencies of 16,400 to 43,600 pg EROD-EQ/g SPMD. SPMDs from the site 0.5 km downstream of the refinery outfall induced MFO in fish cells, but extracts were one-twentieth to one-fiftieth as potent as effluent-exposed SPMDs. Polyaromatic hydrocarbons (PAHs) and mono-, di- and trimethyl-PAHs were abundant in extracts of SPMDs from the effluent, and to a lesser degree, in SPMDs from 0.5 km downstream. It is unknown at the present time which of the compounds in the SPMDs caused the MFO induction. Although SPMD extracts induced fish cells, and chemistry data showed many PAHs in refinery effluent, small rainbow trout exposed to effluent showed little MFO response. Fish exposed to effluent for 4 d showed only three-fold induction over control fish, which was less than seen in tests of other refinery effluents.

A REVIEW OF ENVIRONMENTAL CONTAMINANT LEVELS IN TRADITIONAL FOOD IN NORTHERN CANADA

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ABSTRACT

The potential health effects of environmental contaminants in traditional food has become a concern among northern communities because of the presence of environmental contaminants in the Arctic ecosystem. Over the last ten years, there has been considerable effort to monitor the level of contaminants in fish and wildlife collected from different regions in northern Canada. A large amount of data on many species across the country is available. We have conducted an extensive literature review on levels of environmental contaminants in Northern Canada. The ranges of levels of four contaminants of major concern (chlordane, Hg, PCB and toxaphene) in 59 species of marine mammals, terrestrial mammals, birds, fish and plants are summarized. This data represents 58% of the 101 species of fish, wildlife and plants mentioned in our dietary interviews conducted in the northern communities. Mathematic modelling of the distributions of the data showed that contaminant levels in most food groups are log-normally distributed and have a typical coefficient of variation of about 100%. An example of using the mathematic model for dietary exposure assessment is presented. With the current knowledge of environmental contaminant levels in the northern traditional food system, it may be feasible to conduct preliminary risk assessment of dietary exposure of environmental contaminants when some diet information for a community is available.

INTRODUCTION

Environmental contaminants such as organochlorines and heavy metals are found in the Arctic environment as a result of long-range atmospheric and oceanic transport and local mining activities (Barrie et al., 1992). Potential health effects on Indigenous Peoples are a concern because humans are at the top of the food chain and some of these pollutants are known to bioaccumulate (Lockhart et al., 1992; Muir et al., 1992). Over the last ten years, there has been considerable efforts to monitor the level of contaminants in fish and wildlife collected from different regions in northern Canada (DIAND). A large number of data in many species across the country is available. We have conducted an extensive literature review on levels of environmental contaminants in Northern Canada. Sources of data include commercial, private and government data bases (1986-1995) as well as our in house analytical results. The data were also classified systemically so that they can be easily used for dietary assessment. The aim of the exercise is to build a mathematic model using the existing contaminant data for estimation of dietary exposure level.

RESULTS AND DISCUSSION

Over 20,000 data items were identified in over 50 published articles, unpublished data, government reports and review articles. Ranges of levels of 4 contaminants (chlordane, Hg, PCB and toxaphene) in 59 species of marine mammals, terrestrial mammals, birds, fish and plants collected from 4 geographical regions (Yukon, Mackenzie, Keewatin, Baffin and Northern Quebec) are summarized (Tables 1-4). Data were grouped into seven major traditional food groups including marine mammal meat, marine mammal blubber, terrestrial mammal meat, terrestrial mammal organ, fish, birds, and plants. Marine mammal blubber was separated as an individual group because of their relatively high concentrations of organochlorines. These data represent 58% of the 101 species of fish, wildlife and plants mentioned in our dietary interviews conducted in the northern communities (Table 5).

Samples were collected from almost 800 locations across northern Canada including northern Quebec. Data are available for 61 out of a total of 79 communities in the Yukon and the Northwest Territories.

Table 1. Mercury in traditional marine and terrestrial foods (mg/kg).

Food groups		N	N	Mean±SD	MaX % >0.5
Mar. mammal meat	32	764	0.852±1.05	33.4	32
Mar. mammal blubber	6	71	0.08±0.047	0.130	0
Terres. mammal meat	6	19	0.0.27±0.022	0.166	0
Terres. mammal organ	14	254	0.857±0.900	3.06	64
Fish	799	31441	0.457±0.515	12.3	40
Birds	24	216	0.380±0.594	4.4	13
Plants	8	14	0.015±0.016	0.051	0

Table 2. PCB in traditional marine and terrestrial foods (mg/kg).

Food groups	N sites	N samples	Mean±SD	Max	% >action level
Mar. mammal meat	20	64	0.080±0.165	0.390	0(2)
Mar. mammal blubber	48	554	1.90±2.33	26.6	33(2)
Terres. mammal meat	11	66	0.006±0.005	0.021	0(0.2)
Terres. mammal organ	4	7	0.010±0.008	0.039	0(0.2)
Fish	76	1152	0.052±0.089	2.6	<1(2)
Birds	52	126	0.290±0.893	8.66	<1(0.5)
Plants	13	17	0.006±0.008	0.030	0

Table 3. Chlordane in traditional marine and terrestrial foods (mg/kg).

Food groups	N sites	N samples	Mean±SD	Max
Mar. mammal meat	17	30	0.032±0.036	0.066
Mar. mammal blubber	42	381	1.16±1.20	6.46
Terres. mammal meat	6	27	0.034±0.059	0.4
Terres. mammal organ	4	7	0.003±0.003	0.008
Fish	32	533	0.009±0.008	0.19
Birds	16	81	0.002±0.002	0.007
Plants	-	-	-	-

Table 4. Toxaphene in traditional marine and terrestrial foods (mg/kg).

Food groups	N sites	N samples	Mean±SD	Max
Mar. mammal meat	4	7	0.011±0.01	0.046
Mar. mammal blubber	11	192	3.49±3.89	28.9
Terres. mammal meat	8	18	0.001±0.001	0.005
Terres. mammal organ	2	5	-	-
Fish	38	466	0.113±0.081	4.01
Birds	2	3	0.002±0.002	0.003
Plants	-	-	-	-

Table 5. Contaminant database of traditional foods in the Canadian Arctic.

Food groups	No. of species	No. of species	Percentage
Mar. mammals	4	4	100
Terres. mammals	20	14	70
Birds	24	12	50
Fish	22	16	73
Plants	31	13	42
Total	101	59	58

Table 6. Contaminants in a Dene diet.

Food groups	Food g/day	Hg µg/day	Chl	PCB	Tox
Mar. mammal meat	0	0	0	0	0
Mar. mammal blubber	0	0	0	0	0
Terres. mammal meat	205	5.5	0.7	1.2	0.2
Terres. mammal organ	23	20	0.1	0.3	-
Fish	80	13	0.7	4.2	9.0
Birds	8	1.0	0.0	0.2	0.0
Plants	2	0.0	0.0	0.0	0.0
Total	318	39	1.5	6.0	9.3
Previous estimate		-	0.7	4.2	1.1
TD (55 kg)		39	2.8	55	11

Table 7. Contaminants in an Innuit diet.

Food groups	Food g/day	Hg µg/day	Chl	PCB	Tox
Mar. mammal meat	199	170	6.4	16	2.2
Mar. mammal blubber	30	2.4	35	57	104
Terres. mammal meat	147	4.0	5.0	0.9	0.2
Terres. mammal organ	1	0.9	0.0	0.0	-
Fish	42	6.6	0.4	2.2	4.8
Birds	2	0.8	0.4	0.0	0.0
Plants	2	0.0	0.0	0.0	0.0
Total	423	185	47	76	111
Previous estimate		122	30	53	62
TD (55 kg)		39	2.8	55	11

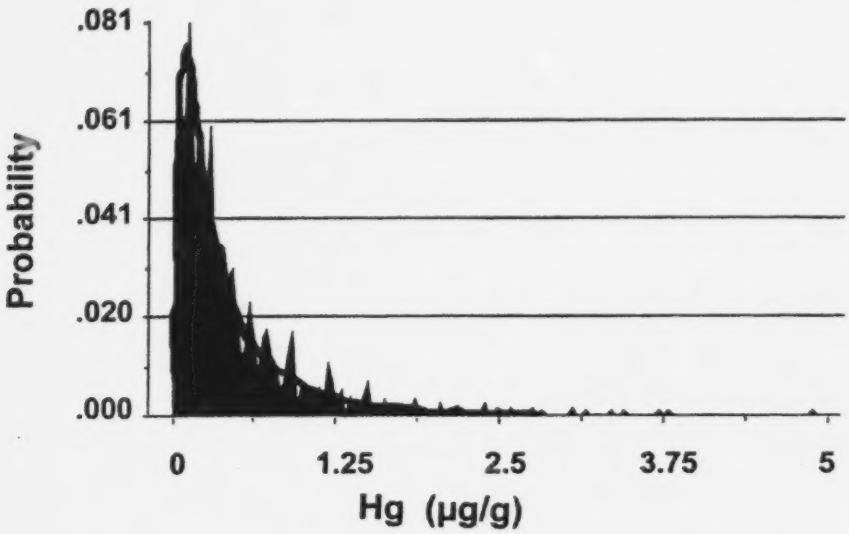

Fig. 1. Distribution of mercury in traditional diet.

Most data available for fish and marine mammals were generated by the Department of Fisheries and Oceans and those of birds by the Canadian Wildlife Service. The arithmetic mean, standard deviation and maximum value of all data in each food group were calculated. Distribution of the data includes inter-species as well as geographical variations. However, the coefficient of variations of the four contaminants in all the food groups ranged between 59% to 300% with a mean of 120±52%. Therefore, values between the plus/minus 100% of the mean would be a reasonable estimation of the range of contaminant levels.

Using the distribution-fitting software of Crystal Ball (Version 4.0, Decisioneering, Inc., Boulder, CO, USA), we observed that lognormal distributions usually adequately describe the contaminant levels, with the Kolmogro-Smirnov statistic for goodness-of-fit tests often less than 0.07 and usually less than 0.15. Results of distribution analysis for Hg in fish is presented here as an example (Fig. 1). Similar distributions were observed for the other contaminants (data not shown).

Over 30,000 data are available for Hg in fish (Table 1). Most of them are for samples collected from northern Quebec. High level of Hg is found in fish from this region because of methylation of Hg in the impounded reservoirs as a result of the hydro-electric projects. Forty percent of the fish data exceeded the guideline level of 0.5 mg/kg. However, if data from northern Quebec is excluded, only 2% exceeded the guideline level. Thirty two percent of the data on marine mammal meat exceeded 0.5 mg/kg. However, of the four marine mammal species included in the database, seal, walrus, narwhal and beluga, seal meat is by far the more popular meat. Seal meat has relatively lower Hg level; only 4% of seal meat exceeded 0.5 mg/kg. Sixty-four percent of data on terrestrial mammal organ exceeded 0.5 mg/kg. We cannot find any guideline level for Hg in organs. The guideline level of for fish may not be applicable as higher percentage of mercury in mammal organ exists as inorganic Hg than fish.

Highest level of PCB was observed in the marine mammal blubber (Table 2). Thirty percent of the data exceeded the guideline level for fish at 2 mg/kg established by Health Canada. Less than one percent of fish data exceeded the guideline level. Less than one percent the data on bird exceeded the guideline of 0.5 mg/kg for poultry on a fat basis. Therefore, the consumption of marine mammal blubber will remain as a concern in terms of PCB contamination. Because of their lipophilic nature, highest

levels of chlordane and toxaphene were found in marine mammal blubber (Tables 3 and 4). Elevated levels were also found in fish.

A typical daily contaminant intake level can be calculated by multiplying the food intake data (g/d) to the mean concentration of the food group (µg/d). We use the dietary data of two of our previous studies conducted in Fort Good Hope/Colville Lake and Broughton Island (Chan et al., 1995; Kuhnlein et al., 1995) to estimate the intake level of PCB and Hg in a Dene diet. Results are presented in Tables 6 and 7. The Dene diet contains mainly caribou meat and fish and no marine mammals whereas the Inuit diet contains large portion of marine mammal meat and blubber. Intake levels were compared to the tolerable daily intake level (TDI) of 0.05 µg/kg/d for chlordane, 0.2 µg/kg/d for toxaphene, 1 µg/kg/d for PCB and 0.71 µg/kg/day (derived from the PTWI of 5.0 µg/kg/wk) for total Hg established by Health Canada. Assuming the body weight for women is 55 kg, the daily intake level of all 4 contaminants except for toxaphene are within the guideline level for the Dene diet (Table 6). However, all contaminant intake levels were higher than the guideline level for the Inuit diet. These results agreed with data of our previous reports. Moreover, the rough estimations of the intake level in this study fall within the standard deviation of the mean values. These result suggest that the database establish will be useful for the estimation of intake level when some diet information for a community of a region is available.

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SOURCES AND FATE OF CONTAMINANTS IN THE GREAT SLAVE LAKE, NT

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Long Range Atmospheric Transport (LRTAP) has been identified as a major source of pollutants into the Arctic. Great Slave Lake, is Canada's fourth largest body of freshwater, it is located in south central

Canadian Arctic (latitude 61-63°N and longitude 109-119°W). Great Slave Lake in turn feeds into the Mackenzie River, Canada's largest river flowing to the Arctic Ocean. Unlike most Arctic lakes which are glacially fed, Great Slave Lake receives a large input of water from Slave River. The Slave River originates in Alberta, where there is substantial industrial activity and development is taking place. Due to the high surface to volume ratio, Great Slave Lake has the potential to act as a large catchment for atmospheric contamination. In this study Air, precipitation, water, and suspended sediments samples were collected from 6 locations within the separate zones of the Great Slave Lake along with samples from the Slave and Mackenzie rivers. All samples were analyzed for organochlorine pesticides including toxaphene, PCBs, PAHs, and heavy metals. Results of this study and a mass balance model will be presented.

ENVIRONMENTAL TOXICOLOGY
SESSION CHAIR: R. ROBINSON

THE EFFECT OF TREATED DOMESTIC SEWAGE EFFLUENTS ON THE SURVIVAL AND GROWTH OF POLYCHAETE *Neanthes arenaceodentata*

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A 7 d toxicity test was conducted using primary, secondary and combined domestic wastes (50:50) on the survival and growth of the juvenile polychaete *Neanthes areanceodentata*. Five test concentrations, each with 10 worms in five replicates, of each effluent type were used with concentrations ranging from 18 to 100%. Sea water and sea salts, used to adjust salinity, were employed as controls. The 7 d LC_{50} was 70% and 88% for primary and combined effluents, respectively. Survival was over 80% in the 100% secondary treated wastes. Growth was compared to a pre-weight group of worms, and the data were recorded as dry weight growth per worm per day. All worms died in 85 and 100% primary treated wastes, negative growth (=loss of weight) was recorded at 50%, and was less than control growth at 18%. Negative growth occurred with worms exposed to 100% secondary treated wastes and was less than control at 18%. All worms died at 100% combined effluent, negative growth occurred at 85% and reduced growth was recorded at 18%. This test is useful for monitoring domestic sewage effluents, it could be used to demonstrate the improvement or degradation of sewage treatment. By increasing the number of worms used in a treatment, it can be used to determine the bioaccumulation of contaminants in the tissue. Extending the test period for a longer period of time could measure the effect of the effluent on the production of gametes and reproduction.

RAPID SIMULTANEOUS QUANTITATIVE AND QUALITATIVE ANALYSIS OF ORGANIC MATTER IN SEDIMENTS BY NEAR-INFRARED SPECTROSCOPY

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Organic matter content of sediments is routinely used to normalize metal and organic contaminant concentrations across sediments of varying types. In contrast, the character of the organic matter is rarely analyzed in contaminant studies even though it is expected to greatly influence metal availability and bioavailability. We have found that near-infrared reflectance spectroscopy (NIRS) is a rapid

technique for simultaneous quantitative and qualitative analysis of organic matter in sediments. NIRS is useful for determining organic C, N, and P and possibly S, in seston and in finely-ground or coarse freshwater sediments. NIRS detects absorbence of light energy by O-H, C-H, C-N and other bonds in the 700-2500 nm area of the electromagnetic spectrum. Widely used in many industries and research areas, the application of NIRS to environment is an emerging field. In addition to elemental analysis, coupled with principal component analysis, NIR provides information on the chemical nature of the organic matter. Wavelengths in the NIR spectra heavily weighted statistically in the first several principal components can be identified as, e.g., protein, lipid, cellulose and so on. This indicates chemical components of the organic matter associated with variability in the constituent being analysed. The use of NIR involves considerable up-front effort to develop calibrations. Nonetheless, it potentially can greatly improve efficiency and cost-effectiveness of routine quantitative and qualitative analysis of organic matter.

MODELLING METAL INTERACTIONS AT FISH GILLS

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The working model of metal interactions at fish gills used in my laboratory is one in which the metal cation interacts with negatively charged sites on fish gills. Once bound to the gills, a metal can exert its toxic effects directly (e.g. ionoregulatory and respiratory disturbances) or once internalized. Competition from other cations, such as Ca^{2+}, for negative binding sites on the gill decreases metal binding and therefore metal toxicity to the fish. In addition, complexation of metal cations by dissolved organic matter (DOM) reduces the amount of metal available to bind at the gills, also reducing metal toxicity. This approach is not new, but our determination of metal-gill binding constants to model metal toxicity to fish is a novel approach. Essentially, we determine the strength of metal binding (e.g. silver-gill binding constant is log K = 10.0) by running ligand exchange experiments using metal binding ligands of known binding srength (e.g. silver-thiosulphate with a binding constant of log K = 8.8). Metal-gill binding constants are then inserted into powerful aquatic chemistry programs such as MINEQL+ or MINTEQ, to predict metal bindings to gills - and therefore metal toxiicty to fish - in water of any given chemistry. Especially important are our determinations of metal-DOM binding constants, because DOM is the major complexing agent of metals in freshwater, This modelling method is powerful, but needs further verification, and its applicability to other aquatic animals needs to be investigated.

IS BIOASSAY THE APPROPRIATE PARADIGM FOR AQUATIC TOXICITY TESTS?

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Lethal, and other quantal, toxicity tests clearly owe a great debt to classical bioassay but we need to ask if this is an appropriate paradigm. The purpose of a classical bioassay is to estimate the potency of an unknown relative to a standard. The necessary assumptions for this estimation are that, in the standard, the response is actually due to the substance of interest, and to this alone, and that the unknown can be viewed simply as a dilution or concentration of the standard, having no active ingredient except the substance of interest. These assumptions lead directly to the classical parallel line assays for the estimation of potency, this method focuses on the LC_{50} as the 'best' point at which to compare materials.

The purpose of an aquatic toxicity test is to characterize the toxicity of the material of interest. In most aquatic toxicity tests the material under test will be an industrial effluent which is a complex mixture of potentially deleterious compounds and there will be no standard material available for comparison.

Because the effluent is a complex mixture the bioassay assumption of a linear relation of the transformed response to the transformed dose concentration cannot be supported. In addition the characterization of toxicity by the LC_{50} alone cannot be justified, an estimate of the slope (variance) is also required and the form of the response function must also be considered.

Regulations of effluent discharge seem to accept some zone of deleterious effect, otherwise they would require that any deleterious effect of the undiluted effluent would result in sanctions. It seems possible, therefore, to invert the usual way of thinking and require that at a dilution of δ the proportion killed (affected) be not greater than π. A definition of this nature leads to a very simple experiment which is much easier to perform than the standard dose-response test and which should result in 'better' estimates. The difficulties in defining δ and π are not be denied, but some pragmatic decisions seem to have been taken by the practical regulators, the people actually in the field enforcing regulations.

FISH KILL RELATED TO SPILL OF CHLORPYRIFOS

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The crash of a spray plane near Melfort, Saskatchewan on July 27, 1994, resulted in the release of a small quantity of the insecticide chlorpyrifos into Melfort Creek upstream of the Melfort Reservoir. Approximately 10,000 dead fish (mostly suckers with a few walleye and pike) were discovered in the upper reaches of the reservoir shortly after the spill. Water samples collected from Melfort Creek on July 28, 1994, showed high levels of chlorpyrifos (11,000 µg/L) at the crash site with levels ranging from 0.1 to 16 µg/L in the creek at various locations downstream. Tissue analysis on fish collected from Melfort Reservoir on August 2, 1994, showed detectable levels of the insecticide. A second set of water samples collected August 3, 1994, showed chlorpyrifos concentrations of 5.6 µg/L at the crash site in Melfort Creek, with values ranging from 0.5 to 2.9 µg/L downstream in the creek throughout the reservoir. These values exceed LC_{50} levels for many species of invertebrates and fishes. Another fish kill occurred on August 10, 1994, involving about 100 fish, this time mostly walleye. Dissolved oxygen monitoring indicated that a significant oxygen depletion had not occurred at the time of the fish kill.

Additional monitoring on October 20, 1994, indicated that chlorpyrifos was still present in the water and sediments of Melfort Creek, 85 d following the spill. Levels in water ranged up to 2.2 µg/L, and in sediment up to 17 µg/kg. The last sampling survey was conducted on September 20, 1995, and showed no residue of chlorpyrifos remaining in the water or sediments of Melfort Creek or Melfort Reservoir. Based on these studies, the insecticide chlorpyrifos demonstrated extreme toxicity and greater persistence in water and sediment than suggested by available literature.

ECOLOGICAL ENDPOINTS
SESSION CHAIR: R. ROBINSON

DETERMINING CRITICAL EFFECT SIZES FOR STREAM BENTHIC INVERTEBRATES IN SOUTHERN ONTARIO

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Surveys of stream benthos are commonly used to evaluate the effects of anthropogenic activities on stream ecosystems. Use of any ecological endpoint as a managerial tool depends on knowing how much change in that endpoint can be tolerated while still maintaining our ecosystem-level goals. Proposing that any change is unacceptable has flawed logic because detection of a statistical difference is dependent on sample size. Rather, it is important to be able to specify a non-zero effect size that we consider to be an important (ecologically relevant) effect. We propose that ecologically relevant effects on stream benthos are those effects that correspond with ecologically relevant changes in fish community composition. Further we define ecologically relevant effects on fish as those effects exceeding 2X the within-reference-area standard deviation. We demonstrate that the responses of fish and invertebrate communities to ecosystem stresses are highly correlated, and that impacts on benthos can be used to predict the degree of impact on fish communities. Benthic community composition changes less than fish community composition to anthropogenic stressors because there are a higher number of taxa. Consequently, impacts on benthic communities between 1 and 2X the within-reference standard deviation tend to reflect ecologically relevant effects on fish communities, critical benthic effects depend on collection methods and taxonomic level of the survey. Generally, benthic surveys at the Family level do as good a job of predicting impacts on fish as do surveys at the Generic or Species levels. Because of shorter life cycles, benthos respond faster to changes in environmental condition. Consequently, the results of benthic surveys allow us to speculate on whether fish community composition is likely to become degraded or improve in the future.

COMPARISON OF A SENTINEL SPECIES EVALUATION FRAMEWORK TO A FISH COMMUNITY APPROACH FOR ASSESSMENT OF SMALL STREAM FISH WITHIN AN URBAN STREAM

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Streams located within urbanized areas boast features that are absent or reduced in streams found in more natural areas such as episodic urban storm-water runoff, reduced channel heterogeneity, increased impoundment, barriers, and reduced in-stream refugia for fish. The magnitude of these alterations make ecological assessments of urban streams difficult and limits our ability to identify and interpret critical factors influencing the biota. The effects of urbanization on fish occurring in the predominantly urban Laurel Creek were assessed using two approaches: community structure analysis and a sentinel species evaluation framework. Fish communities and a sentinel species (creek chub (*Semotilus atromaculatus*); Cyprinidae) were sampled at 13 sampling locations from headwaters to confluence of Laurel Creek with the Grand River. Hierarchial cluster analysis, diversity, and evenness measures showed strong associations with upstream barriers (e.g. reservoirs), the urban core and associated barriers (e.g. weirs), and barrier-free extreme downstream sections. Compared to historical data, numerous species have reduced distributions or have been extirpated downstream of the reservoirs and below the urban core. Intolerant species like blacknose shiner (*Notropis heterolepis*) and

rainbow darter (*Etheostoma caeruleum*) have been replaced by more tolerant and fecund species like common carp (*Cyprinus carpio*) and brown bullhead (*Ameiurus nebulosus*). Longitudinal community composition shows a shift to species tolerant of controlled flows, sedimentation, and high water temperatures. These features, in conjunction with barriers impeding recolonization result in reduced diversity and evenness in the community. Population characteristics of creek chub collected downstream of the reservoirs and below the urban core showed similar condition, reduced growth rates, lower fecundity, smaller absolute sizes, elevated levels of atresia (failed ova development), increased age of first reproduction and greater maximum age compared to fish collected upstream of the reservoir in more pristine areas. The sentinel species response pattern is consistent with fish responding to a reduction in preferred food resources due to degradations in water quality or a niche shift, and exposure to sub-lethal contaminant(s). Except for the response to sub-lethal contaminant(s), both the fish community approach (using the ecological tolerances and requirements of species) and the sentinel species response pattern identify the same environmental stressors contributing to the success of these fish.

USE OF CHIRONOMIDS AS BIOINDICATORS OF CONTAMINANT STRESS

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Environmental stresses produce effects ranging from subtle biochemical changes that protect an organism from damage, through sublethal developmental effects that interfere with growth and compromise fitness, to death. Chironomids' benthic habits, short life cycle and unique developmental and genetic structure make them excellent biological indicators of stress. We evaluated short and long-term responses of *Chironomus* larvae to laboratory and field exposures to Cd and PAHs. Chironomid abundance in mesocosms was reduced by 65% or more by exposure to nominal creosote concentrations of ≤1.7 ml/L. In laboratory bioassays, creosote-spiked sediments produced a 48-h LC_{50} value of 1.4 ml/kg sediment. In the laboratory, *Chironomus riparius* larvae were inoculated into sediments spiked with benzo-[*a*]-pyrene (BAP, up to 10,000 mg/kg) or Cd (up to 10,000 µg/kg). Larval survival and incidences of mentum deformity were unaffected by BAP, but exposure to 625 µg/kg Cd doubled mortality and significantly raised incidences of mentum deformity. Cd-exposed larvae exhibited significantly enlarged Balbiani Rings of the 4th chromosome in salivary gland preparations within 12 h, indicative of increased transcription of RNA precursors to salivary mucoproteins. Exposure to sublethal Cd concentrations (40 µg/kg) delayed development, eventually producing larger pupae that exhibited reduced emergence success. Deformed larvae also exhibited reduced emergence success. Our results confirm the potential of chironomids as short, medium and long-term bioindicators of contaminant stress.

USING THE FREE-ION ACTIVITY MODEL TO PREDICT ANIMAL CADMIUM CONCENTRATIONS IN NATURE

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General theoretical models that reliably predict the bioaccumulation and effects of pollutants, are required for the effective management of contaminated ecosystems. One such tool is the free-ion activity model. This model has proven useful in predicting metal accumulation and toxicity to aquatic organisms. It postulates that the biological effects of metals are best predicted by the activity of the free metal ion, rather than by total metal concentrations. Since this model was developed in the laboratory under unnatural experimental conditions, its validation in field situations is a prerequisite to proclaiming

its general applicability in nature. We show that this model successfully explains cadmium concentrations in an insect (*Chaoborus punctipennis*) collected from a large series of lakes. We show that successful prediction depends on the explicit consideration of cadmium complexation with dissolved organic matter, as well as competition between free cadmium and hydrogen ions for biological uptake sites. Our results suggest that the free ion model would provide an effective theoretical framework for the use of animals as sentinels of metal contamination in nature.

MONITORING CONTAMINANT TRENDS IN FISH: FACTORS OTHER THAN LOADINGS

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Programs that monitor trends in environmental contaminant levels routinely expect that temporal changes in biological community burdens are a direct response to similar changes in external loadings to the system. Proposals to either strengthen pollution abatement legislation or relax the regulations are often based on the direct assessment of temporal trend toxic chemical monitoring data. Unfortunately over time the factors effecting the uptake and accumulation of contaminants in biological communities may have changed significantly. Therefore, the assumption that trend through time ecosystem contaminant burden data are directly correlated with trends in loadings would be false. In order for valid assessments of long term data to occur, changes over time of all factors influencing contaminant accumulation must be identified and their impact on valid trend evaluations either eliminated or minimized. The influence of changes in simple variables such as fish age, sex, lipid content and time or season of collection, which affect contaminant accumulation patterns, has been determined. The impact of other more complex factors such as shifts in biological community composition, diet, alterations in lipid chemistry and perhaps even climate, is more difficult to quantify. A review of the associated long term biological community observations is a most important activity related to the validation of temporal trend contaminant burden datasets. These datasets often contain incidental information on community form and function which is key to the analysis of chemical information. Additionally activities such as retrospective analysis of stable isotope levels in archived samples plus a more detailed analysis of chemical constituents in historical collections often provides information on changes to the functioning of the foodweb over time which effect the accumulation of contaminants and therefore trend data.

ACCURACY OF IMMUNOASSAY SCREENING METHODS

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INTRODUCTION

Immunoassays are useful semi-quantitative tools for sample screening. They may be used in conjunction with a quantitative analytical method such as one using gas chromatography. Samples may be first screened using the immunoassay, and only those samples found to contain analyte need be analyzed by the quantitative method. This may result in considerable cost savings. Of course, it is important that the accuracy of the immunoassay method relative to the quantitative method be acceptable, and that the number of 'false negatives' is acceptably small.

Interpretation of immunoassay results for the purpose of sample screening typically involves comparing

the absorbance reading of the sample to that of a standard. A sample absorbance greater than that of the standard indicates that the sample is 'negative' relative to that standard, whereas a sample absorbance which is less than that of the standard indicates that the sample contains analyte, and is 'positive.' Consideration of the error, i.e. the standard deviation of the absorbance reading allows the assignment of a sample as 'borderline', indicating that it should be considered as positive and be tested by the quantitative method for confirmation. This work investigated the accuracy of immunoassay tests relative to quantitative methods. Soil and water samples were analyzed for several analyte groups including PAH, PCP, and TEH. The focus was on 'false negatives.'

Interpretation of results

Seventy six samples of soil and water were tested using immunoassay kits, and also using quantitative (GC) methods, as per EPA protocols. The immunoassay result for each sample was assigned as one of those in Table 1 based on [1] the absorbances of the sample and standard, [2] the error of these absorbances, [3] the concentration of analyte in the sample observed using the quantitative (GC) method, and [4] the error of the quantitative method.

Table 1. Possible immunoassay results.

Result	Abbreviation
Positive	+
Borderline positive	~t
False positive	FP
Borderline false positive	~FP
Negative	-
False negative	FN
Borderline false negative	~FN

These immunoassay results were devised considering nine possible combinations of immunoassay absorbance and GC concentration (analyte concentration observed using the GC method) as shown in Figs. 1, 2 and 3. The key for these figures is listed in Table 2. In Fig. 3 for example, the immunoassay absorbances ("X") are all significantly greater than that of the immunoassay standard, suggesting that these 3 samples contain little or no analyte. The 3 possible GC concentrations are shown in these charts. The chart labelled 'FN' shows a GC concentration significantly greater than that of the immunoassay standard, making this sample a 'false negative' (FN). But if the GC concentration fell within the vertical dashed lines (being not significantly different from the concentration of the immunoassay standard), the immunoassay result was downgraded to a 'borderline false negative, (~FN).

Table 2. Key for interpretation graphs in Figs. 1, 2 and 3.

Graph element	Description
Horizontal line	Absorbance of IA standard
Horizontal dashed lines	Error of absorbance
Diagonal line	IA standard curve
Vertical line	Concentration of IA standard
Horizontal bar	GC concentration
Vertical dashed lines	Error of GC concentration
"X"	IA absorbance

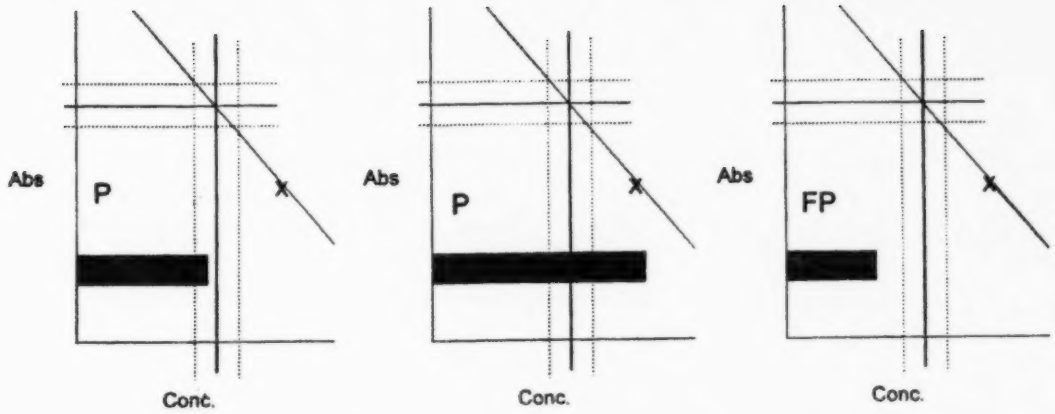

Fig. 1. Immunoassay sample absorbance significantly less than that of standard.

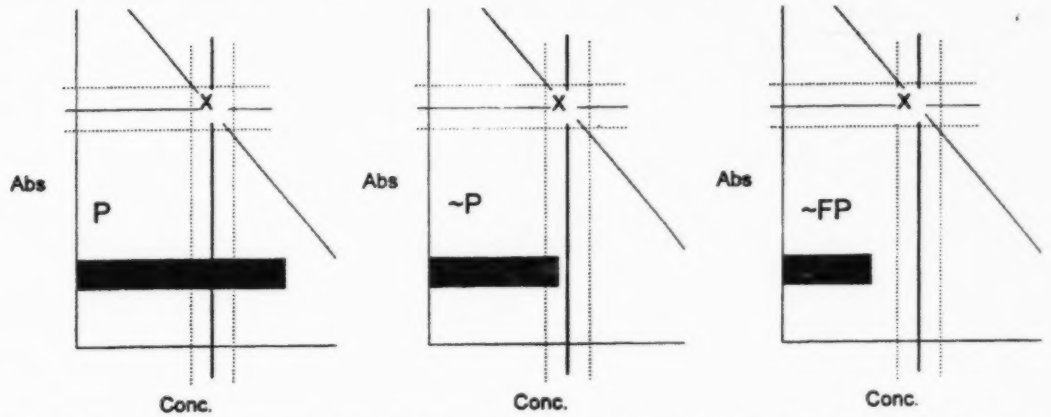

Fig. 2. Immunoassay sample absorbance not significantly different from that of standard.

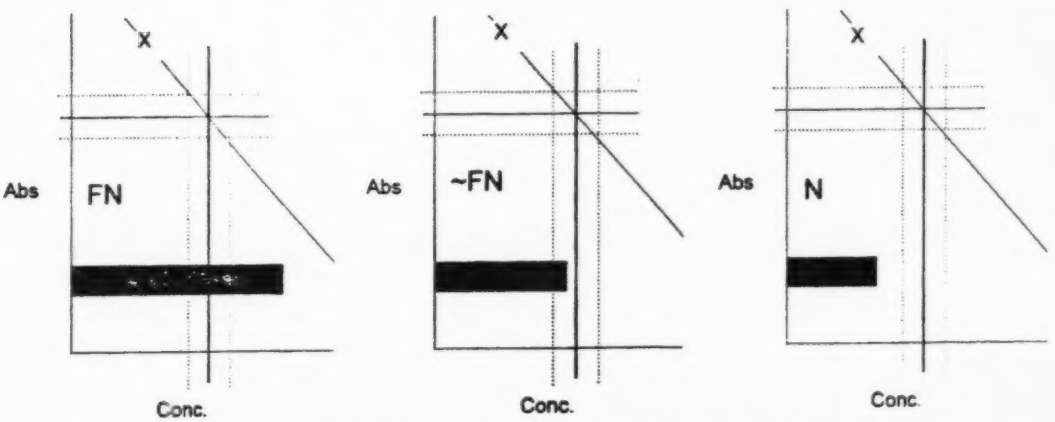

Fig. 3. Immunoassay sample absorbance significantly greater than that of standard.

In Fig. 2 the immunoassay sample absorbances are all within the horizontal dashed lines, and are not significantly different from those of the standard. Such samples were labeled as 'positive', 'borderline positive', or 'borderline false positive', depending on the GC concentration. Fig. 1 shows the possible immunoassay results when the sample absorbance is significantly less than that of the standard. The relative error for the immunoassay absorbance (which defined the space between the parallel dashed lines) was varied and the effect that this had on the numbers of false negative results was examined. The error of the GC concentrations was kept constant at 10%.

RESULTS

A total of 76 samples were tested by immunoassay and quantitative (gas chromatographic) methods. The numbers of samples for each matrix and analyte are listed in Table 3. The accuracies of the immunoassay results are shown in Fig. 4. Varying the error of the absorbance of the immunoassay standard had the following effects. [1] Using an error of 10%, there were 2 false positives (3%), and 6 false negatives (8%). Note that all false negatives were soil samples; no water samples were false negative. [2] Reducing the immunoassay error to 0%, as would be the case when simply comparing sample and standard absorbances using the kit instructions, increased the number of false negatives to 9 (12%). [3] Using a very large immunoassay error (80%), 3 (4%) false negatives were still observed. These might be called 'severe' false negatives, where the absorbance was much greater than that of the standard.

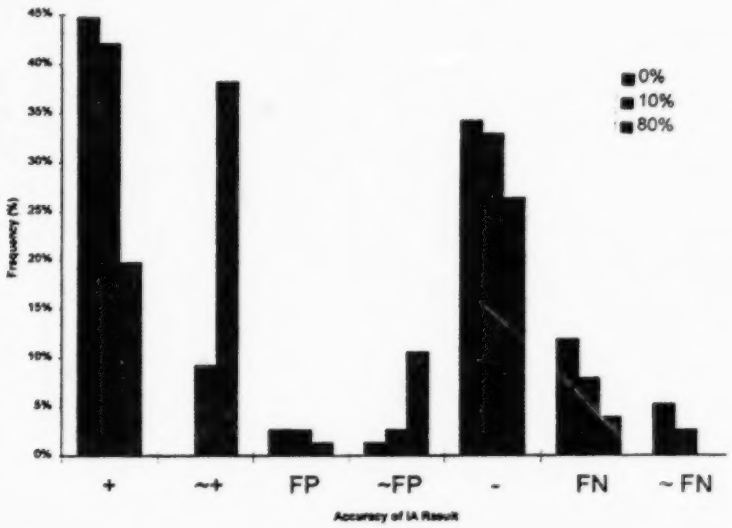

Fig. 4. Numbers of positives (+), borderline positives (~+), false positives (FP), borderline false positives (~FP), negatives (-), false negatives (FN), and borderline false negatives (~FN), at different immunoassay absorbance errors.

Table 3. Analytes and matrices tested.

Matrix	Analyte	No. of samples tested
Soil	Total petroleum hydrocarbons (TPH)	6
Soil	Polycyclic aromatic hydrocarbons (PAH)	33
Soil	Pentachlorophenol (PCP)	16
Water	Pentachlorophenol (PCP)	20

CONCLUSIONS

By considering the error of the immunoassay absorbance reading, the number of false negatives was reduced by downgrading some of them to 'borderline false negative.' Four percent of the samples were considered as 'severe' false negatives. These (2) soil samples were taken from different geographical regions and were tested for different analytes. This suggests that false negative samples may not always be the ones with absorbances close to that of the standard, but may also have significantly high absorbances (relative to the standard), and that some of these samples should be tested using the quantitative method for confirmation.

ENVIRONMENTAL FATE OF PESTICIDES IN WETLAND COMMUNITIES

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Wetlands are important areas for water control as well as repositories of biodiversity. Wetlands can also function as environmental sinks for toxic contaminants. Persistent organic contaminants in wetlands can cycle through wetland food webs and show ecosystem level effects many years after their application. In this study, insects, fish, amphibians, reptiles, and small mammals were collected from a large freshwater marsh and analysed for chlorinated pesticides. DDE was the dominant chemical contaminant detected in all organisms. Reptiles accumulated the highest tissue concentrations of DDE, due in part to their high trophic position in the wetland community and their lack of an effective chemical elimination mechanism relative to aquatic organisms. A positive relationship was demonstrated between DDE accumulation in amphibians and the degree of terrestrial habitat use by these species. Species extirpations in this marsh were greater than could be explained by the species-area relationship. The historic application of organic chemicals, trophic transfer of these compounds, and the life histories of specific wetland organisms contribute to the magnification of chemicals in organisms and the potential for extinction of sensitive wetland species.

ATMOSPHERIC ORGANOCHLORINE BIOACCUMULATION PATTERNS IN A ROCKY MOUNTAIN AQUATIC FOOD WEB

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In 1991-92, 14 lakes in the Canadian Rocky Mountains were surveyed for organochlorine contaminants (PCB's, DDT isomers, toxaphene, and other pesticides) in water and lake trout. Lake trout from Bow Lake, a glacier-fed lake near the Continental Divide in Banff National Park, contained particularly high concentrations of toxaphene when compared to other mountain lake trout populations. Other organochlorines, particularly DDT and PCB's were also detected in the fish and invertebrates. It was hypothesized that the contamination was due to increased biomagnification through the food web. This hypothesis was tested by analyzing lake trout (*Salveninus namaycush*), mountain whitefish (*Propsopium williamsoni*), benthic invertebrates, and zooplankton for organochlorine contaminants. Stable nitrogen and carbon isotope ratios were used to characterize trophic interactions. Lipid content of organisms was determined. Based on the stable isotope data, both fish species were found to be invertebrate eaters with lake trout feeding on both zooplankton and benthic invertebrates, and mountain whitefish feeding exclusively on benthic invertebrates. Lake trout and their zooplankton prey had higher organochlorine concentrations and higher lipid proportions than those found in mountain whitefish and their benthic prey

species respectively. In this lake, the main variable in organochlorine bioaccumulation in fish appear to be the composition of a fish's diet instead of its trophic position in the food web.

FURTHER ADVENTURES IN THE ANALYSIS OF AQUATIC MESOCOSM DATA: ORDINATION TECHNIQUES

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While the current paradigm developed by the U.S. EPA for ecological risk assessment discourages the use of mesocosm studies, there are circumstances under which data from controlled field experiments are valuable. These include investigating whole-system responses, predicting biotic responses to perturbations in complex systems, and verifying risk assessments. It is imperative, therefore, that appropriate methods for analysis and assessment of aquatic mesocosm data be advanced. These should focus on multivariate approaches because they retain and incorporate the spatial and temporal multidimensional nature of biological communities. We provide empirical data to illustrate the use of ordination techniques in evaluating the effects of novel forest pesticides on zooplankton communities in lake enclosures. The importance of community-level studies and environmental realism in ecological risk assessment is discussed.

OVERVIEW AND ASSESSMENT OF THE RISK FROM CHEMICAL CONTAMINANTS TO FISH, FISHERIES RESOURCES AND FISH HABITAT IN BRITISH COLUMBIA AND THE YUKON

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A regional assessment conducted for the Department of Fisheries and Oceans - Pacific Region was used to determine the risk from chemical contaminants to selected fish stocks, fisheries resources and fish habitats in British Columbia and the Yukon. The assessment was conducted by evaluating published scientific information, and through discussions with local scientists. Recommendations were made for possible mitigative actions, and for future monitoring requirements. The methodology employed was adapted from a protocol developed by the International Council for the Exploration of the Sea (ICES, 1988). Information collected was ranked using a modified form of the risk rating index of Pierce and Morrison (1991) which assigns a numerical rating based upon the degree of exposure to a chemical contaminant. Ratings range from '0' indicating a risk "known not to be a problem due to previous research" to '5' indicating a "known interaction with deleterious effect on fish stock, on fishery for that stock, on a key food web link supporting an important fish stock, or on a critical habitat supporting an important fish stock." Results showed that urbanized areas expose fish stocks to the greatest risk by virtue of urban and industrial contamination. Industrial areas in isolation of urban development may also pose a risk to fish stocks. Atmospheric transport was found to be a significant source of contamination in some northern areas. Certain areas of Vancouver Island and the Fraser River Basin exhibited the highest risks to fish stocks, many of which were associated with urban and industrial centres.

ICES. 1988. Report of the International Council for the Exploration of the Sea Advisory Committee on Marine Pollution. ICES Cooperative Research Report No. 160.

Pierce, R., and J. Morrison. 1991. A risk index for contaminant hazards to fish and fisheries. Draft report. Oceanography and Contaminants Branch Physical and Chemical Sciences Directorate, Department of Fisheries and Oceans, Ottawa, ON.

Figure 1.
DFO Pacific Region Divisions.

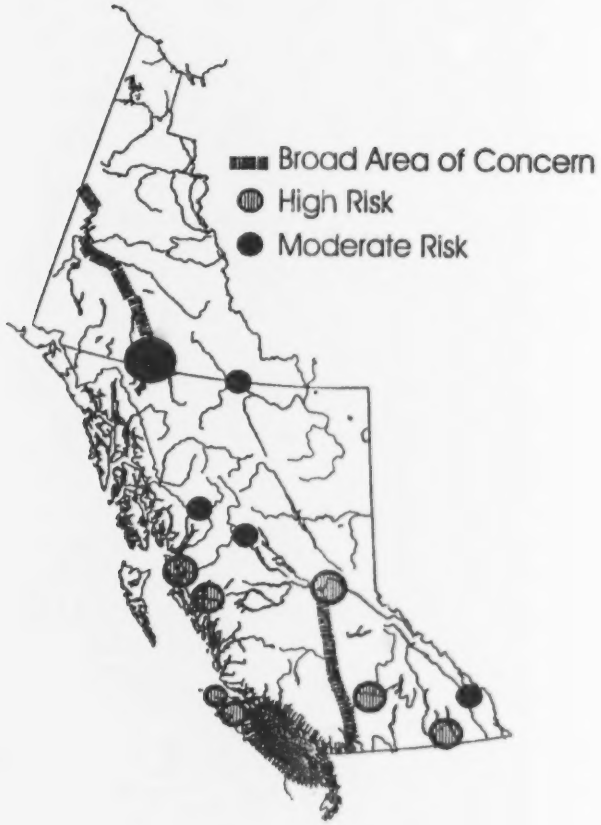

Figure 2.
Areas of High and Moderate Risk
and of Broad Concern

NEW DEVELOPMENTS IN TOXICITY TEST AND RISK ASSESSMENT METHODS
SESSION CHAIR: R. SCROGGINS

ACUTE TOXICITY ASSESSMENT OF INDUSTRIAL EFFLUENTS WITH A MICROPLATE-BASED *Hydra attenuata* ASSAY

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The acute toxicity potential of ten industrial plants located in Toyama Prefecture (Japan) was appraised with a microplate-based assay developed with the freshwater cnidarian *Hydra attenuata*. Three measurement endpoints (LC_{50}, EC_{50} and TC or 'threshold concentration') were determined based on specific morphological changes displayed by *Hydra* under conditions of progressive intoxication. Four effluents were shown to be lethal toward *Hydra* while eight induced sublethal toxicity responses. LC_{50}s varied from 18.8 to 100% v/v, while EC_{50}s ranged from 15 to 100% v/v. Similarly, lethal and sublethal TCs ranged from 17.7 to 100% v/v and from 8.8 to 100% v/v, respectively. Statistical analyses performed on all toxicity data for the ten effluents confirmed that the sublethal endpoints (EC_{50}s and sublethal TCs), previously unreported to assess complex wastewaters, proved to be more sensitive than the lethal endpoints (LC_{50}s and lethal TCs). This was also reflected by lethality to sublethality ratios which ranged from 1 to 2.6 (LC_{50}/EC_{50} comparisons) and from 1.1 to 5.4 (LC_{50}/TC comparisons) within a 96 h exposure period. A correlation was also observed between conductivity and *Hydra* responses highlighting a possible link to the presence of toxic metal ions. Based on our study, this simple and cost-effective microassay appears valuable as a (sub)lethal toxicity screening tool for effluents. Additional studies are planned with chemicals and other environmental matrices to better circumscribe its scope of usefulness.

RESEARCH AND DEVELOPMENT OF A RAPID AGGREGATION INHIBITION BIOASSAY USING THE HETEROTROPHIC FLAGELLATE, *Polytomella papillata*

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An aggregation inhibition bioassay using the heterotrophic flagellate, *Polytomella papillata*, has recently been used in the assessment of the toxicity of snake venoms (Gittleson, 1994). The test method involves assessing the relative time that it takes for the test organism to aggregate in an exposure test tube, in a series of toxicant concentrations. The duration of the proposed test exposure is rapid, and does not require any microscopy for cell enumeration, an obstacle for many microscale tests. An obvious extension of this bioassay is as a tool in evaluating environmental toxicants. We developed this test in a toxicological context further by [1] standardizing the details of culture maintenance of *P. papillata*, and to enhance cultures for testing, [2] examining the reproducibility of performing the test with the same toxicant on different occasions using different batch cultures, and [3] developing and refining the standard operating procedure (SOP). Sodium chloride and cadmium nitrate, were investigated as potential reference toxicants; both inhibited the aggregation behaviour of the flagellates, however, $CdNO_3$ was more sensitive than NaCl in inhibiting this response. Tests were also conducted using a pulp and paper mill effluent, the aggregation response appeared to be enhanced, rather than inhibited, by exposure to the effluent. A full description of the test method, and the results generated during the study, performed to date will be presented. Recommendations for future work with the test method, including method standardization, and testing with other environmental toxicants will be discussed.

DEVELOPMENT OF A 7-DAY EMBRYO TEST FOR RAINBOW TROUT

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Short-term toxicity tests that evaluate chronic toxicity and/or effects on critical life history stages are widely used to evaluate ambient and effluent water quality. However, these test have largely focused on warm-water species, primarily the fathead minnow, which generally have short life cycles. Conversely, salmonids generally exhibit greater sensitivity and may be the species of interest in coldwater habitats and in streams that serve as spawning areas for anadromous stocks. Over 30 tests were conducted that evaluated embryo viability from the point of fertilization through the onset of morphogenesis. The tests were evaluated in terms of control performance, sensitivity, and variation in reference toxicant results. The data indicated that the procedures are robust (no control failures), sensitive (MSDs averaged 15 percent of the control) and reproducible (relatively little variation in reference toxicant results). The endpoint, embryo viability, is easily recognizable and can be observed without the use of a microscope. The testing requirements, in terms of glassware and sample volume, are similar to those required for the 7 d larval fathead minnow test.

RECENT LABORATORY STUDIES RELATED TO IMPROVING ENVIRONMENT CANADA'S BIOLOGICAL TEST METHOD EPS 1/RM/28 USING RAINBOW TROUT EMBRYOS ("E" Toxicity-test Option)

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One of a series of biological test methods published by Environment Canada's Method Development and Application Section includes Report EPS 1/RM/28 which describes three test options for measuring the toxicity of aqueous solutions to early life stages of salmonid fish. Studies summarized here looked at improving procedures and conditions for the shortest test option, a 7 d embryo ("E") test which starts with freshly-fertilized rainbow trout eggs. The influences of test duration (7 or 10 d) and test temperature (12 or 15°C) were evaluated for developmental rate, mean percentage of non-viable control embryos, and sensitivity to a reference toxicant (zinc sulphate). Also investigated were the effects of differing test apparatus (using differing test volumes, two setups with embryo-incubation units and one without) on performance and sensitivity of the E test using control water and a range of concentrations of biotreated pulpmill or papermill effluent. Final recommendations for the E test are a 7 d test at a temperature of 15±1°C using a simple-to-construct incubation unit in a 4 L capacity plastic pail, with an improved aeration system, and a reduced (2 L) volume of test solution. Subsequent to test termination, replicate groups of embryos and unfertilized eggs are scored as the number and percentage of non-viable embryos. The appropriate statistical endpoint is the EC_{50} based on the mean percentage of non-viable embryos for each treatment after adjustment for the percentage of non-viable control embryos using Abbott's correction.

THE GRASS SHRIMP EMBRYO-LARVAL TOXICITY TEST: A SHORT-TERM PREDICITIVE BIOASSAY

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ABSTRACT

A new short-term predictive bioassay for estuarine and marine organisms is described. The toxicity test uses embryos of the estuarine grass shrimp *Palaemonetes pugio*. Embryos were exposed to sublethal

concentrations of selected pesticides. The protocol essentially involves single pulse exposure of the embryos to sublethal concentrations of the toxicant and seawater or acetone control for four days followed by transfer into uncontaminated seawater for the rest of the embryonic development. After hatching, the following toxicity endpoints were measured at the larval stage: morphological abnormality (frequency of occurrence and severity), hatchability (% hatch) and larval viability for 4 d post hatch. Hatchability was the least sensitive in most cases and morphological abnormality the most sensitive. The grass shrimp embryos exhibit differential response to the herbicide, lasso; the insect growth regulator, diflubenzuron; the organophosphate insecticide, chlorpyrifos; and the organochlorine insecticide, kepone.

Morphological abnormalities (terata) observed were: stuntedness and ballooning of the larvae at hatching; loss of setae or segments of the cephalic and appendages; thoracic curvature of the abdomen and telson. Abnormal larvae were also shown to exhibit abnormal swimming behavior and the inability to feed, which ultimately resulted in high mortality. For all four pesticides, and for each toxicity endpoint, there was a concentration-response relationship. Also, these abnormalities were shown to correlate well with the survivability of the larvae (larval viability).

The maximum acceptable toxicant concentration (MATC) for each parameter was determined. The ecological significance of the results together with the potential use of the grass shrimp embryo-larval toxicity (GSELTOX) test as a short-term predictive test is discussed.

INTRODUCTION

The embryonic and larval stages have long been recognized as the most sensitive life stages of fish (Macek and Sleight, 1977; McKim, 1985). Toxicity tests with these life stages have been used to estimate the maximum acceptable toxicant concentration (MATC) within a factor of 2 in most cases (McKim, 1977). Since then, early life stage (ELS) tests for fish have been developed which emphasizes evaluation of sublethal effects. Many of these test were developed as methods for estimating chronic toxicity (Norberg, 1985; EPA, 1994). Similar trends hold for few marine invertebrates and mysids are the most well studied. A 7-d survival growth and fecundity test with *Mysidopsis bahia* has been adopted by US EPA for estimating chronic toxicity of effluents to marine and estuarine organisms (EPA, 1994). A similar protocol is being developed using *Mysidopsis intii* (Langdon et al., 1996).

The grass shrimp *Palaemonetes* (species) have been used extensively in toxicity testing (Buikema et al., 1980). Much of these test are acute toxicity tests. Presently there is no standard short-term test with the grass shrimp for estimating chronic toxicity despite the wealth of information on the general biology and response of different life stages of this crustaceans to environmental pollutants (e.g. see McFarland and Pickens, 1965; Wood, 1967; Welsh, 1975; Tyler-Schroeder, 1978; Wilson, et al., 1985; Wilson and Costlow, 1986a; 1986b; Douglass et al., 1992). This fact together with the ease with which this shrimp can be reared in the laboratory, their ubiquitous occurrence and their role in estuarine food webs, make the grass shrimp an excellent candidate for the development of short-term tests.

Wilson (1985) has described a type of ELS test for*Palaemonetes pugio* which was called delayed sublethal bioassay (DSB). Essentially the DSB protocol involved 4 day exposure of the grass shrimp embryos to sublethal pollutant concentrations and monitoring the delayed effects produced at the larval stage. This test method was shown to be predictive of chronic exposure of grass shrimp to diflubenzuron (Wilson, 1985; Wilson et al., 1995). Kirby-Smith et al. (1989) used caged gravid female *P. pugio* in a modified version of the DSB protocol to test the toxicity of agricultural runoff. The results indicated that larvae obtained from exposed embryos had significantly low survival (27-35% compared with the controls (94-98%). Chemical analyses of the runoff water also indicated that the pesticide concentration was 8 nanograms. Thus grass shrimp embryos can be sensitive to very low pesticide concentrations even in field conditions. Fisher and Foss (1993), used a grass shrimp embryo hatching test methodology adapted from protocols of Wilson (1985) and Middaugh et al. (1988) to determine

toxicity of No. 2 fuel oil in seawater. The results indicate that the test was very sensitive, highly reproducible and with very low variability. Similar test sensitivity was reported by Mearns et al. (1995) for effects of bioremediation treatment after an intentional oil spill in Delaware Bay. In fact hatching of shrimp embryos was more sensitive than either the Microtox® or sea urchin fertilization tests (Mearns et al., 1995). Clearly there is now a growing interest in the use of grass shrimp embryos for monitoring estuarine pollution.

This paper describes a new short-term predictive bioassay using the grass shrimp embryos and larvae. This test named GSELTOX (grass shrimp embryo-larval toxicity) test is almost identical to the DSB method of Wilson (1985) with few modifications. The test is developed primarily as a subchronic toxicity test for marine and estuarine pollution chiefly from toxic organics (namely pesticides). However, from the results of other researchers cited earlier, this test can be applied to many different situations and for a wide range of chemical pollutants. The ecological relevance of results from this test is discussed.

MATERIALS AND METHODS

Ovigerous shrimp were obtained either from a laboratory 'habitat' or were collected from the field and acclimated for at least 4 d in the laboratory. The shrimp were sorted according to stage of embryonic development (based on yolk consumption and eye development) as described in Wilson (1985). Shrimp carrying 6-d old embryos (Stage 4, body segmentation) were selected for the tests. Embryos were carefully dissected from the pleopods of 2 or 3 females (using micro-dissecting instruments) and placed in 8 cm diameter Carolina culture bowls containing filtered (to 0.45 μm) sea water at 20 g/L. Either Instant Ocean sea salts or natural sea water was diluted with de-ionized water to 20 g/L.

The pooled clutches of embryos were rinsed 2 times with sea water (20 g/L) before transferring to the test solutions. Embryos were randomly assigned, using a large-bore disposable pipette, to 125 ml Pyrex® Erlenmeyer flasks containing 50 ml seawater or test solutions until 20 embryos were in each flask. The flasks were covered with parafilm and placed on a platform shaker in an environmental chamber set at 25∘C and 12:12 h photo-period. The shaker speed was set at 150 rpm because unpublished data on hatching rhythm of dissected grass shrimp embryos showed that shaking at this speed resulted in synchronous hatching and ≥ 90% hatching success compared to <5% for embryos in still water. The embryos were exposed for 4 d to the same test solutions (i.e. single pulse 4-d exposure) as described in Wilson (1985). After 4 d all the embryos were transferred to clean flasks containing 20 g/L filtered (to 0.45 μm) seawater and returned to the shaker. During the transfer, care was exercised to keep contamination to a minimum. The flasks were shaken continuously until the eggs hatched.

The embryos were monitored daily using a binocular dissecting microscope to determine embryo survival, embryo abnormalities, hatching and larval abnormalities. The swimming behavior of the larvae was also noted. This is because it has been reported that the photo-behavior and swimming speeds of *P. pugio* larvae were altered when embryos were exposed to sublethal DFB concentrations (Wilson, 1985; Wilson et al., 1985; Wilson et al. 1987). For ease of observation, the content of each flask was poured into 8 cm diameter Carolina culture dish. Such handling had no effect on the development and hatching of the embryos. Indeed, results of another study indicate that hatching success increased with agitation. Differences in swimming behavior between normal and abnormal larvae were pretty obvious. While normal larvae swam actively in the water column (invariably upside down and with the telson leading the way), abnormal larvae often exhibited everything from erratic uncoordinated movements to mere twitches when the larvae barely drag along the bottom of the bowl. In very severely abnormal larvae, hardly any movement was noticeable. For correlation of larval abnormalities with swimming and vertical distribution in *P. pugio* as a result of embryonic exposure, see Wilson et al. (1987). After hatching and hatching success determined, the larvae were reared in seawater in 8 cm diameter Carolina culture dishes for 4 d i.e. until the first molt. This usually occur on the third day at 25∘C. During this time the larvae were fed freshly hatched *Artemia salina* nauplii. The percent survival to day

4 (viability to first molt) was determined. This marks the end of the test which last a total of 9 d. The test method described here is a modification of the delayed sublethal bioassay (DSB) suggested by Wilson (1985) and was used to determine the effects of sediment on toxicity and persistence of diflubenzuron (Wilson et al., 1995). This modified test is renamed the grass shrimp embryo-larval toxicity (GSELTOX) test. Total test duration can be reduced by using 8-d old (embryonic-eye stage) and 10-d old (compound eye stage) embryos. Survival to 4-d post hatch was used as an abbreviated larval viability measure. This is a good estimator of larval survivability to post-larvae because detailed studies with diflubenzuron (Wilson, 1985) and Lasso (unpublished data) show that larvae hatched from exposed embryos and survive the first molt (i.e. to the 2nd larval stage) usually have higher survival rate to the post larval stage. Also, for diflubenzuron, larvae from exposed embryos exhibited abnormal phototaxis. This abnormal behavior persisted while the larvae molted to the 2nd larval stage when normal photoresponse was restored (Wilson et al., 1985).

The main test chemicals and concentrations used were: chlorpyrifos (seawater and acetone control, 0.25, 0.5, 1.0, 2.0, and 4 µg/L); diflubenzuron (seawater control, 0.3, 0.5, 1.0, 2.5, and 5.0 µg/L). Kepone (seawater and acetone control, 0.3, 0.5, 1.0, 2.5, and 5.0 and 10.0 µg/L) and Lasso (seawater control, 0.5, 1.0, 3.0, 5.0, and 10.0 mg/L). All test concentrations were nominal. These compounds were selected to represent different classes of pesticide. Chlorpyrifos is an organophosphate, diflubenzuron an insect growth regulator, kepone an organochlorine insecticide and Lasso a herbicide.

Statistical analyses were performed on the data using one way analysis of variance (ANOVA) followed by Sums of Squares Simultaneous Test Procedure (SSSTP) to determine significant differences between treatments (Sokal and Rohlf, 1981). The level of significance was set at $p<0.05$ throughout the test. No observed effect concentrations (NOEC) and lowest-observed effect concentration (LOEC) values were determined for the different toxicity endpoints studied. Acceptability of the test was set at $\geq$ 80% survival of embryos and larvae, and no larval abnormality in the controls.

RESULTS AND DISCUSSION

Photomicrographs of the different stages of grass shrimp embryonic development together with the mean ages when reared in the laboratory at 25±1°C are shown in Fig 1. Embryonic development proceed normally in all controls and very low test concentrations of the chemicals. However, at the highest test concentrations, gross embryo malformations and significant reduction in embryo survival were observed. A selection of some commonly encountered embryo malformations are given in Fig. 2. Other malformations (photos not shown) include: Irregular shape embryos; embryos with amorphous or diffuse yolk; 'hour-glass' embryos, where embryos are constricted in the mid-section by withdrawal from the egg membranes; swollen embryos- where embryos swell up to 50% normal size of controls and unaffected embryos of the same age in the same flask; embryos with yolk and embryonic eye depigmentation and yolk disintegration. During depigmentation, the eye pigments appeared bleached and undergo somewhat gradual color changes from black to brown and on to reddish brown. Similarly, (depending on the type of chemical) the yolk undergoes disintegration in which in addition to color changes, the integrity of the yolk is gradually lost until an amorphous mass of oil globules is scattered through the eggs or appear as large clumps. It should be noted that malformed embryos always die before hatching or fail to hatch successfully (i.e. mortality is always 100% for such embryos).

Exposed embryos without obvious malformations develop until hatching but the larvae may exhibit gross morphological malformations most of which have been reported for DFB toxicity (Wilson, 1985; Wilson et al., 1995; Wilson, 1997). A simplified method for quantifying the severity of larval abnormalities is given in Table 1. A more accurate method for quantifying the larval abnormalities involves morphometric measurements and application of formulae derived for two abnormality indices (see Wilson, 1985; Wilson et al., 1995 for details). Abnormal larvae are easily discernible when compared with control larvae under the microscope and the descriptions given in Table 1 is adequate for scoring severity without actually measuring them. This method of scoring abnormality in the larvae has been

Table 1. Simplified method for quantifying severity of terata (abnormalities) in > 1 day post hatch *P. pugio* larvae during GSELTOX test.

Score	Severity	Description of terata
0	None	No morphological abnormality observed. Larvae with complete setation on all appendages; telson symmetrical and 6th abdominal segment straight.
1	Slight	Very mild ballooning and stuntedness but no loss of setae or curvature of abdomen. Size of larvae not significantly different from controls.
2	Moderate	Stuntedness; ballooning; loss of some setae on cephalic and thoracic appendages or telson. No abdominal curvature or flexed telson.
3	Severe	Severe stuntedness and ballooning; loss of most or all setae from appendages; abdominal curvature and/or flexed telson; telson may be folded laterally (butterfly-wing condition) or dorso-ventrally ('eagle-claw condition').
4	Very severe	As for 3, but in its extreme condition. e.g. many segments of appendages absent; abdomen twisted into spiral (corkscrew condition). Commer-shaped larvae; curved rostrum; complete loss of telson.

tested successfully with both graduates and undergraduate students over the last 9 years. Very little training time was required for the students to grasp the technique. Fig. 3, are photomicrographs showing details of the 3 regions of the larvae from control (A) and exposed (B) embryos. The severity of larval abnormalities were always concentration - dependent for all the chemicals tested to date. Whole mounts showing normal, severe and very severely abnormal larvae are presented in Fig. 4. For the four pesticides tested, larvae with slight abnormality usually exhibited 4-d viability similar to the controls (Tables 2-5), however, larvae with severity of abnormality score ≥2 resulted in 4-d viability that was significantly lower than the controls (except for Lasso). These results are similar to that reported by Wilson (1985) for DFB and grass shrimp embryos when the larvae were reared to the post-larval stage. Thus there is direct correlation between larval abnormality and survivability of the larvae. This makes the results from GSELTOX test more ecologically significant. In one field test for the toxicity of agricultural runoff, exposed *P. pugio* embryos produced larvae with survival of only 27-35% compared with 94-98% in controls (Kirby-Smith et al., 1989). Similarly, direct exposure of *P. vulgaris* larvae to mercury produced morphological deformities in late stages, together with reduced survival to the postlarval stage (Shealy and Sandifer, 1975).

Table 2. GSELTOX test with Chlorpyrifos. Comparison of different toxicity endpoints.

Endpoints		Concentration (µg/L)						
		SW[a]	AC[b]	0.25	0.5	1.0	2.0	4.0
Embryo survival (%)		96	95	96	94	90	90	0
% Embryo malformed		0	0	0	0	0	100	100
% Larvae malformed		0	0	0	94	98	100	ND[c]
Severity of terata		0	0	0	1-2	3	3	4
% Hatch		95	98	96	94	94	88	0
Larval viability (% H-D4)[d]		98±1.7	99.2±1.8	83.2±5.8	12.8±4.1	0	0	0
Larva behavior-swimming	Yes	Yes	Yes	No	No	No	ND	

[a] SW = Seawater control; [b] AC = Acetone control; [c] No data due to 100% embryo mortality; [d] H-D4 = Survival of larvae to 4 d post hatch.

Table 3. GSELTOX test with diflubenzuron. Comparison of different toxicity endpoints.

Endpoints	Concentration (µg/L)					
	SW[a]	0.3	0.5	1.0	2.5	5.0
Embryo survival (%)	99	100	96	98	96	90
Embryo malformed (%)	0	0	0	0	0	0
Larvae malformed (%)	0	0	100	100	100	100
Severity of terata	0	0	1	1.7	3	4
% Hatched	99.7±0.3	100	100	99.3±0.7	98.9±1.1	97±±1.1
Larval viability (%, H-D4)[b]	97.2±1.1	99.2±1.1	98±2.4	75.4±9.0	30.8±12.3	0
Larval behavior-swimming	Yes	Yes	Yes	Yes	No	No

[a] SW = Seawater control; [b] H-D4 = survival of larvae 4 d post hatch.

Embryo survival and hatchability were not affected for all 4 pesticides and test concentrations, except for Lasso at 10 mg/L. Similarly, embryo malformation was not as sensitive as larval abnormality, swimming behavior and 4-d larval viability. Although DFB did not produce any embryo malformation even at the highest (5.0 µg/L) test concentration (Table 3), the other pesticides did (Tables 2, 4 and 5). The type of embryo malformation was not chemical-specific but exhibited strong concentration-response relationship. The most commonly encountered embryo abnormalities were: shrinking of embryo from egg membrane, which is usually accompanied by some swelling; sloughing of outer membrane; and premature hatching. Other forms included yolk disintegration and embryonic eye depigmentation.

Table 4. GSELTOX test with Kepone. Comparison of different toxicity endpoints.

Endpoints	Concentration (µg/L)							
	SW[a]	AC[b]	0.1	0.3	0.5	1	5	10
Embryo survival (%)	96	99	96	95	94	94	92	90
Embryo malformed	0	0	0	0	0	<5	10-15	>20
Larvae malformed (%)	0	0	0	0	93	94	88	95
Severity of terata	0	0	0	0	1	2	3	4
% Hatch	96.2 ±3.9	98.8 ±1.09	96.4 ±3.8	94.5 ±4.6	94.4 ±3.8	94 ±3.8	91.6 ±6.1	89.6 ±2.9
Larval Viability (H-D4)[c]	95.2 ±4.8	98.8 ±1.09	82.8 ±4.15	84.8 ±3.63	82.4 ±8.65	21.6 ±8.88	3.2 ±1.8	0
Larval behavior -swimming	Yes	Yes	Yes	Yes	Some yes	No	No	No

[a] SW = Seawater control; [b] AC = Acetone control; [c] H-D4 = Survival of larvae to 4 d post hatch.

Table 5. GSELTOX test with Lasso®. Comparison of different toxicity endpoints.

Endpoints	Concentration (mg/L)					
	SW[a]	0.5	1.0	3.0	5.0	10.0
Embryo survival (%)	98	98	99	98	95	0
% Embryo malformed	0	0	0	0	65	100
% Larvae malformed	0	0	100	100	100	ND[c]
Severity of terata	0	0	1	2	3	ND
% Hatched	92±1.1	95.6±4.6	98.4±1.7	98.6±2.2	95.2±2.3	0
Larval Viability (H-D4)[b]	97.8±2.3	87.6±4.3	98.8±1.8	95±1.0	73.6±12.9	ND
Larval behavior - swimming	Yes	Yes	Yes	No	No	ND

[a] SW = Seawater control; [b] H-D4 = Survival of larvae to 4 days post hatch; [c] ND = No data, because no larvae hatched.

Table 6. MATCs for 4 test pesticides, using occurrence and severity of terata survivability of larvae to 4 days post hatch and larval swimming behavior.

	Embryo malformation	Larval terata	Intensity of terata	Larval Viability (%, H-D4)	Larval swimming
Chlorpyrifos (µg/L)					
NOEC	1.0	0.25	0.25	0.25	0.25
LOEC	2.0	0.50	0.50	0.50	0.50
Diflubenzuron (µg/L)					
NOEC	ND	0.30	0.30	0.50	1.00
LOEC	ND	0.50	0.50	1.00	2.50
Kepone (µg/L)					
NOEC	5.00	0.30	0.30	0.50	0.50
LOEC	10.0	0.50	0.50	1.00	1.00
Lasso (µg/L)					
NOEC	3.00	0.50	1.00	3.00	1.00
LOEC	5.00	1.00	3.00	5.00	3.00

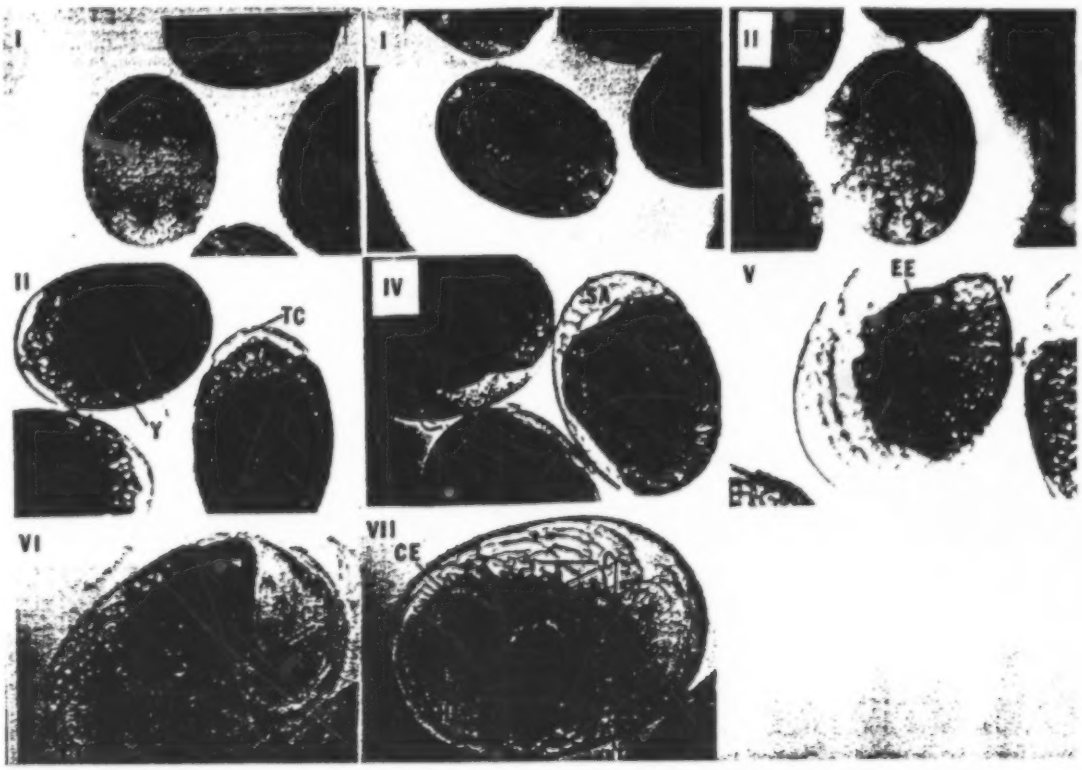

Fig. 1. Photomicrographs of the different stages of embryonic development of *Palaemonetes pugio*. I. Cleavage stage. II. Blastoderm/Gastrula stage. III. Tissue Cap stage. IV. Body Segmentation stage. V. Embryonic Eye stage. VI. Compound Eye stage. VII. Protozoeal stage. Magnification for all photos = 200X. C = chromatophores; CE = compound eyes; EE = embryonic eye; H = head; HT = heart; SA = segmented abdomen; TC = tissue cap; Y = yolk. Photos by J.E.H. Wilson, © 1985.

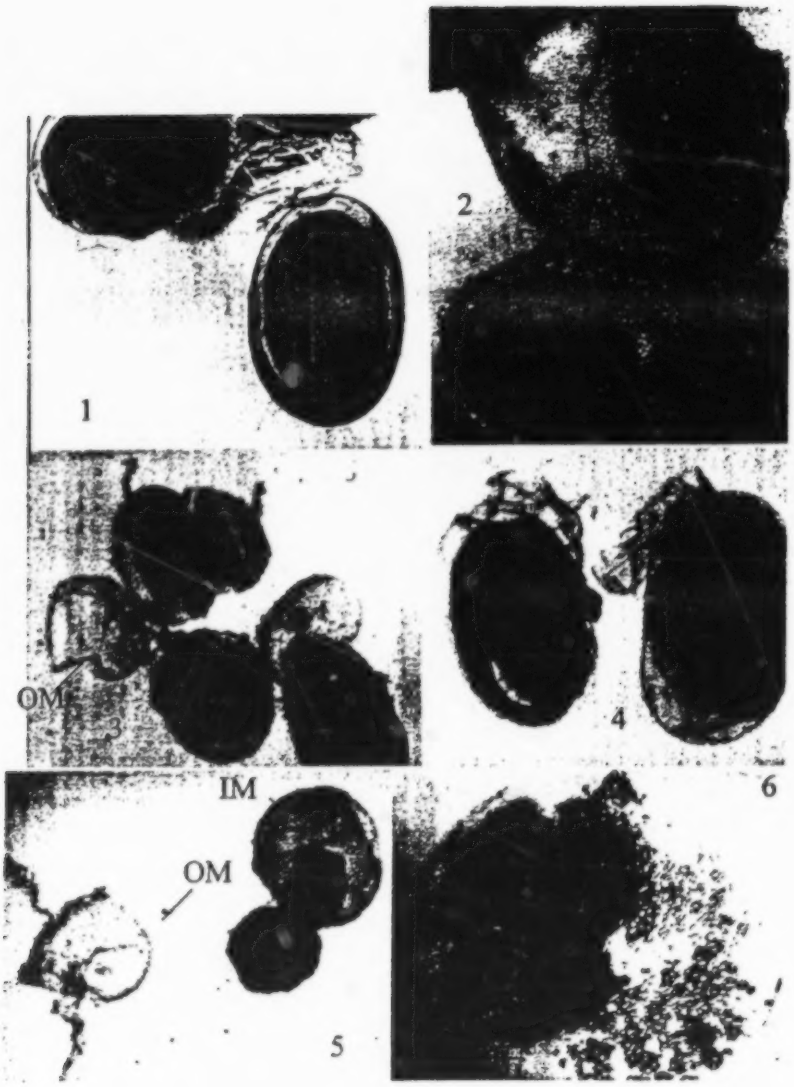

Fig. 2. Photomicrographs of selected embryo abnormalities of *P. pugio* resulting from GSELTOX tests with various chemicals. 1 and 2=Withdrawal or shrinking of embryo from egg membrane. In some, this process is restricted to the middle resulting in an hour-glass appearance (hour-glass embryos); 3=Sloughing of outer membrane in stage VII embryos. Membrane shedding usually precedes premature hatching (hatching in embryos in stages earlier than stage VIII); 4 = Embryo 'trapped' in inner membrane after shedding of outer; 5 = Premature hatching completed, with both outer (OM) and inner (IM) membranes shed. Embryo is seen 'oozing' out of membrane. 6 = Embryo with yolk disintegration in progress. Malformed embryos always fail to complete development. Photos by J.E.H. Wilson © 1995.

Fig. 3. Photomicrographs showing morphological comparison of the different body parts of first stage larvae of *P. pugio* hatched from (A) untreated embryos (B) embryos exposed to 2.5 µg/L DFB. Not distortion of every cephalic appendage and almost complete loss of setae (1B) compared to the control (1A). Similarly the thoracopods in 2B are stunted and devoid of setae seen in the control (2A). The 6th abdominal segment in the exposed larvae (3B) show ballooning and stuntedness (arrow) while the telson shows curvature. Photos by J.E.H. Wilson © 1985.

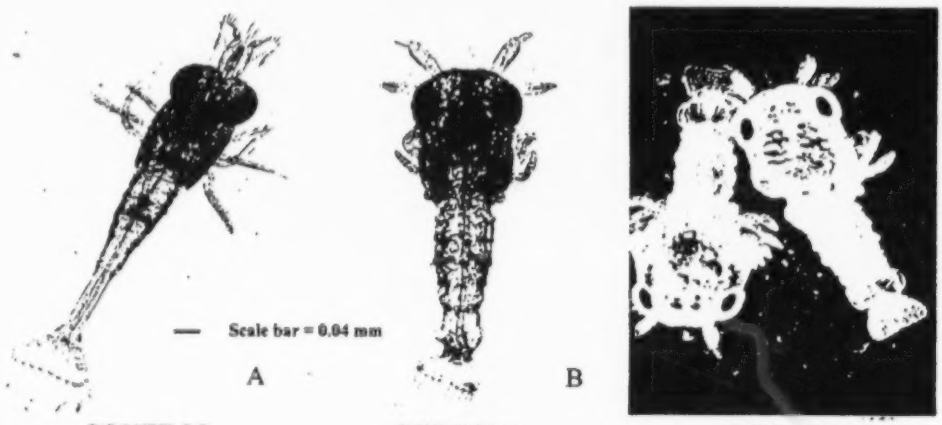

Fig. 4. Whole mounts showing gross morphology of *P. pugio* larvae with no abnormality (A), severe abnormality (B) and very severe abnormality (C). Note the extreme stuntedness and ballooning of C. Photos by J.E.H. Wilson © 1996.

The estimated MATCs for frequency and severity of larval abnormalities (terata), 4-d viability and swimming behavior are similar for each compound tested (Table 6). However, % abnormality was consistently the most sensitive (with lowest MATCs). The advantages of using larval abnormality as subchronic toxicity endpoint together with its possible ecological relevance have been presented in Wilson (1985). Larval abnormality has been shown to correlate positively with survival to the post larval stage. To illustrate the utility of the test, preliminary (i.e. unreplicated) data for 8 other compounds, together with definitive MATCs for the 4 main test pesticides are given in Table 7. This table shows that: [1] a wide range of compounds (different classes) produce teratogenic effects in the larvae when grass shrimp embryos are exposed for 4 days to sublethal concentrations. [2] Although teratogenesis may occur in 85 - 100% of the larvae that hatched from exposure to concentrations > LOEC, the severity of the malformations may or may not be uniform among the larvae at the particular concentration. This appears to be dependent on the type of chemical tested (Table 7), hence the need to test more chemicals. Table 8 shows that the replicability of the GSELTOX test using only larval abnormality and viability to day 4 was satisfactory since the coefficient of variation (CV) was < 41% (excluding the very high test concentrations). The occurrence of larval abnormalities gave even better replication results (CV < 10%). These finding further strengthen the case for GSELTOX as a short-term test for predicting chronic toxicity in estuarine and marine environment.

Table 7. List of compounds that exhibit teratogenesis in *P. pugio* larvae with estimated MATC. Note that except for chlorpyrifos, DFB, Kepone and Lasso, these results are preliminary.

Compound	NOEC	LOEC	Unit	Frequency of teratogenicity
p-Chlorphenylureau	10.0	20.0	mg/L	Quantal, 100%; severity uniform
Chlorpyrifos	0.25	0.5	ug/L	Mostly quantal, ≥85%; severity non-uniform.
Copper Sulfate	0.01	0.1	mg/L	Quantal, 100%; severity varied within concentration.
Diflubenzuron (DFB)	0.3	0.5	ug/L	Quantal, always 100% and with uniform severity (see Wilson, 1985).
Ethyleneglycol	0.01	0.1	%, v/v	Quantal, 100%; severity non-uniform.
Fenoxicarb	0.5	1.0	mg/L	Non-quantal, severity non-uniform.
Kepone	0.3	0.5	ug/L	Mostly quantal, ≥85 severity uniform.
Lasso (hadricide)	1.0	3.0	mg/L	Quantal, 100%; severity non-uniform.
Methylene Chloride	0.01	0.05	%, v/v	Quantal, always 100%, uniform severity.
Potassium dichromate	25.0	50.0	mg/L	Quantal, 100%; severity uniform.
Pro-Drone	0.5	1.0	mg/L	Quantal, 100% and severity is uniform .
Zinc Sulfate	0.01	0.1	mg/L	Quantal, 100%; severity non-uniform.

Table 8. A comparison of coefficient of variation (CV, %) for larval abnormality and viability to 4 day post hatch. All tests had 5 replicates.

Chlorpyrifos			Diflubenzuron			Lasso			Kepone		
Conc mg/L	CV Abn[a]	CV H-D4[b]	Conc mg/L	CV Abn	CV H-D4	Conc mg/L	CV Abn	CV H-D4	Conc µg/L	CV Abn	CV H-D4
SW	NA	1.7	SW	NA	1.1	SW	0	2.4	SW	NA	5.0
AC	NA	1.8	-	-	-	0.5	0	4.9	AC	NA	1.1
0.25	NA	6.9	0.3	NA	1.1	1.0	0	1.8	0.1	NA	5.1
0.5	3.9	32.0	0.5	0	2.4	3.0	0	1.1	0.3	NA	4.2
1.0	1.7	ND	1.0	0	11.9	5.0	0	17.5	0.5	2.1	10.6
2.0	O	ND	2.5	0	39.9	10.0	ND	ND	1.0	6.3	41.2
4.0	ND	ND	5.0	0	ND	-	-	-	5.0	2.3	56.3

[a] Abn = % Abnormal larvae; [b] H-D4 = Survival of larvae to 4 days post hatch; [c] ND = No data because all eggs died prior ro hatching; [d]NA = No abnormality observed; [e] Zero, because abnormality was 100% in all replicates; [1]SW = Seawater control; [2] AC = Acetone control.

The test described here has many advantages including: [1[It is relatively inexpensive in terms of materials and equipment required; [2] It is highly sensitive to a wide range of chemicals; [3] Survival of control embryos is consistently high (≥90%) while larval abnormality is always zero. [4] Larval abnormality (the main toxicity endpoint) has direct ecological consequences for the survival and recruitment of the adult shrimp population. This makes the test results more ecologically relevant. [5] The grass shrimp is ubiquitous throughout the US and can be easily collected and maintained in the laboratory year round for production of embryos. Clearly, the potential application for this subchronic test is very great, but further testing with this protocol is required before it can be recommended for standardization. Some areas needing further study include: Testing with a wider range of chemicals and effluents. Test with standard reference toxicants to determine its repeatability; comparison of this test with other commonly used subchronic toxicity tests for marine pollutants, etc.

SUMMARY

A new subchronic test using the grass shrimp embryo-larval stages is described. This test named GSELTOX (grass shrimp embryo larval toxicity) is being developed as a short-term test for predicting chronic toxicity of pollutants to estuarine and marine invertebrates. A summary of the general test protocol is shown in the Appendix. The test duration can range from 4-9 d depending on the age of embryos used. This test is a modification of the delayed sublethal bioassay (DSB) described by Wilson (1985). Results obtained with GSELTOX for 4 different classes of pesticide indicate that the test reproducibility is very high. Preliminary results with metals, other pesticides, solvents and reference toxicants indicate that this test has the potential for very wide application in aquatic toxicology and risk assessment. These results show that all the toxicity endpoints can be used to estimate the MATCs. Larval abnormality was consistently the most sensitive endpoint. The test uses the most sensitive life stages of the shrimp and single pulse - exposure which may be more representative of short-term field exposure to pesticides and other toxicants. This together with the use of larval abnormality as endpoint make the results from this test more ecologically relevant. This is because abnormal larvae have low viability which could reduce recruitment to the adult population. Another advantage of GSELTOX test is that the results can more easily be extrapolated to predict the chronic effects of anthropogens on commercially important decapod crustaceans such as paeneid shrimp and different types of crabs. Development and evaluation of this test is in progress using standard reference toxicants. To further shorten the test duration and increase its sensitivity, 8- and 10-d old embryos are being tested.

ACKNOWLEDGEMENTS

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APPENDIX

Summary of protocol for grass shrimp embryo - larval toxicity (GSELTOX) test

Parameter	Condition
Test type	Static renewal
Salinity	20±2 g/L
Temperature	25±1∘C
Photoperiod	12h light : 12 h dark
Test container	125 ml Pyrex® glass Erlenmeyer flask
Test volumne	50 ml
Renewal of solution	None during exposure to toxicant; after 4 days transfer all embryos to seawater.
Exposure duration	4 days
Dilution water	Deionized/distilled
Age/Stage of test organisn	At least 6-d old embryo (10-d old for abbreviated version.)
No. of embryos per flask	10-20
No. of replicates	At least 3
Food.	None for embryos. Freshly hatched *Artemia* nauplii for larvae
Areation	None
No. of test concentration	At least 5 and seawater (or solvent) control
Endpoints measured	Embryo survival, hatchability (%); larval abnormality (%); severity of terata; larval survival to day 4.
Total test duration	9 days (4-5 days for abbreviates version.)
Test acceptability	≥80% survival in controls for embryos and larvae; no terata in control larvae.

A ROUND-ROBIN EVALUATION OF CANADIAN SEDIMENT TOXICITY TEST METHODS WITH THE AMPHIPOD *Hyalella azteca* AND THE CHIRONOMID *Chironomus riparius*

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Two biological test methods for freshwater sediment(s) are being developed by Environment Canada using the chironomid *Chironomus riparius* and the amphipod *Hyalella azteca*. The tests run for 10 and 14 d, respectively, and the endpoints measured are percent survival and increase in dry weight (mg) at the end of the test. Both tests may be conducted as either an intermittent renewal assay with twice-daily replacement of the overlying water (as per U.S. EPA 1994) or as a static assay with aeration of the overlying water and no replacement of water. The performance of both test options with both species was compared in an interlaboratory round-robin using sediment spiked with concentrations of Cu (mg\kg d.w.). Participation by both private and government laboratories was voluntary. Results suggest that percent survival is not affected by the use of either option but growth may among laboratories and between the static vs. the renewal system. Coefficients of variation for either system can sometimes be large (>40%) especially for measurements of growth but are generally <30%. LC_{50}'s and IC_{25}'s were usually similar for both the renewal and the static system within a laboratory.

REFINEMENT OF CULTURE CONDITIONS AND NON-CONTAMINANT EFFECTS TESTING USING TWO SPECIES OF SPIONID POLYCHAETES

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Over the past three years, Environment Canada has been actively developing a chronic sublethal toxicity test for survival and growth using Canadian species of marine or estuarine polychaetes. The first phase of the work involved the selection and rating of potential test species from the Atlantic, Pacific, and Arctic coasts of Canada. Fourteen species were chosen for survival and sensitivity testing. Preliminary lab evaluation demonstrated that five species could be collected, held in the lab, and were sensitive to contaminated sediments. The second phase of the project involved culturing trials with five species chosen for further study. During laboratory culturing trials, it was found that two spionid polychaetes, *Polydora cornuta* and *Boccardia proboscidea*, could be cultured over multiple generations in the lab. Contaminant-effect and non-contaminant effect tests were carried out at this time.

Based on results of these two phases, a draft Environment Canada test method using spionid polychaetes was prepared and distributed for review. The review committee identified the following areas which required further work, [1] improvement of culturing techniques, [2] defining the effect of temperature (20 or 23°C) and salinity (5, 10, 15 and 28 g/L) on culture and test performance, [3] further definition of the effects of sediment grain size and organic content, and [4] definition of the tolerance of each species to ammonia. A detailed experimental design has been prepared and work is in progress. Results will be discussed in the context of culture and test conditions thought to be appropriate for Environment Canada's test method using spionid polychaetes.

A SPECIFIC AND SENSITIVE METHOD FOR ACCURATE DETECTION OF ENVIRONMENTAL CONTAMINANTS WITH ESTROGEN-LIKE ACTIVITY

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There is increasing awareness of environmental contaminants with estrogen-like activity that could adversely influence human health and aquatic life. It is possible that these estrogenic compounds contribute to the increased incidence of estrogen-dependent neoplasia as well as abnormal growth, development and reproduction in human and wildlife populations through contamination of various ponds, lakes and rivers. Among the environmentally persistent chemicals with estrogen-like activity are a number of commonly used pesticides and industry-related chemicals such as phthalate esters, butylbenzyl phthalate (BBP), di-*n*-butylphthalate (DBP), butylated hydroxyanisole (BHA), polychlorinated biphenyls (PCBs), and polynuclear aromatic hydrocarbons (PAHs). These compounds have a tendency to bioconcentrate in animal fat, and are difficult to measure by chemical means because of the wide diversity of their molecular structure. For this reason the best approach would be to use an effective bioassay which can detect estrogenic activity. Recent studies in our laboratory have demonstrated that estrogens significantly alter the production of messenger ribonucleic acid (mRNA) for various hormones in the goldfish pituitary. We have clearly demonstrated that very small quantities of these compounds (2 µg/L) significantly increase production of mRNA for growth hormone and gonadotropin hormones above basal levels. These findings indicate that growth and reproduction of fish, birds, and mammals can potentially be influenced by environmental contaminants with estrogen-like activity through entry into the aquatic system.

Our approach for the measurement of environmental contaminants with estrogen-like activity would be to use a very sensitive and specific bioassay in terms of pituitary gonadotropin subunit and growth hormone gene expression in goldfish. This assay can be performed both *in vivo* and *in vitro*. Our *in vivo* approach will be to expose goldfish to contaminated water through immersion or injection for a period of time, followed by removal of pituitary and estimation of gonadotropin and growth hormone mRNA levels. The *in vitro* approach will be useful for more sensitive assays since small amounts of contaminated water can be used to treat cultured pituitary tissue for 5-10 h, followed by estimation of gonadotropin and growth hormone mRNA levels. Overall, we describe here a specific and sensitive method for accurate detection of environmental contaminants with estrogen-like activity based on both *in vivo* and *in vitro* approaches.

DEVELOPMENT OF ECOLOGICALLY-RELEVANT TERRESTRIAL TOXICITY TESTS FOR ASSESSMENT OF CONTAMINATED SOILS IN CANADA

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Most standardized whole-soil test methods developed to assess the toxicity of contaminants to soil organisms have used the approach where a chemical is added to an artificial soil with specific physico-chemical characteristics and the test organisms are exposed to the chemical-soil mixture. Relatively little effort has been devoted to developing test methods for evaluating the toxicity of soils from hazardous waste and other contaminated sites. The notable exception are the test methods developed by the U.S. EPA research laboratory in Corvallis, OR, which are directly applicable to the assessment of contaminated soils.

Environment Canada and the Canadian Association of Petroleum Producers have embarked on a five year program to develop, standardize, and validate a battery of soil toxicity tests using various earthworm, plant, springtail and mite species which can be used to assess the relative toxicity of

contaminants in soils. These tests must be applicable to soil conditions typically found in Canadian environments and test species must be representative of soil invertebrates or plants inhabiting soil ecosystems in Canada. In early 1995, the toxicity test development phase of the program was initiated at the University of Guelph. The experimental test design with each species incorporates two negative experimental controls consisting of an artificial soil and a reference soil which is also used as a diluent of contaminated soils. During the first year, earthworm method development has focused on acute lethality testing using *Eisenia fetida, Lumbricus terrestris,* and *Enchytraeu albidus* while future research will focus on sublethal endpoints. During the same time period, the screening of 30 plant species was initiated and includes species representing six dicotyledonous and two monocotyledonous families. The species of plants with one exception (i.e. *Arabidopsis* sp.) are representative of both above- and below-ground crop plants grown in agricultural lands across Canada, as well as non-crop plants that are also widely distributed in Canada. Endpoints measured in each plant test include root and shoot length and both wet and dry weight. Key results from both method development efforts will be discussed during the presentation.

AN OVERVIEW OF RESULTS OBTAINED USING MICRO-SCALE TOXICITY TEST KITS WITH EFFLUENTS, SEDIMENTS AND CHEMICAL MIXTURES

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Over the past few years the frequency of use of micro-scale test kits has greatly increased. A variety of test kits are being used to test effluents, sediment and chemical mixtures primarily because they are cost-effective and rapid. A distinct advantages of several of these tests is the elimination of culturing organisms, an activity which requires much investment for laboratories. This presentation will review the results of several studies where a total of over 15 mini-tests have been used to test various substances. The endpoints measured include a variety of lethal and sublethal endpoints such as mortality, growth or light inhibition, genotoxicity, test duration varied from 15 min to 72 h. All tests reviewed have been evaluated qualitatively in terms of criteria such as simplicity, rapidity, sample volume needed, cost, sensitivity, correlation with conventional tests, and ecological relevance. Recommendations are made for the commercial use of certain test kits as representative screening tools and for future use ecotoxicological evaluations. Research and development needs will also be discussed.

SEDIMENT TESTING
SESSION CHAIR: P. RIEBEL

HONG KONG SEDIMENT QUALITY VALUES AND DECISION-MAKING

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EXTENDED ABSTRACT

Extensive dredging and disposal operations in Hong Kong have required classification of sediments for decision-making. Specifically, hazard identification is necessary to protect the environment from highly

contaminated sediments and to ensure appropriate management of limited resources such as confined disposal facilities.

Since 1992, sediment hazard in Hong Kong marine waters has been determined by means of sediment quality 'criteria' for seven metals (Cd, Cr, Cu, Pb, Hg, Ni and Zn). These values are generally conservative but subjective in derivation. They have been used to determine three different classes of sediments, ranging from uncontaminated to contaminated; however, differences between sediment contaminant concentrations across the three classes are within analytical variability, ranging from factors of 1.2 to 1.7. Thus these 'criteria' are not as useful as they should be. Further, there are no Hong Kong sediment quality values for other metals, metalloids or organics.

In 1996, the sediment quality values for these seven metals were revisited based on advances in the science and in region-specific information, and the immediate needs of Hong Kong. There are three generic options for assessing contaminated sediments, [1] decision-making based only on sediment quality values (the existing status quo in Hong Kong), [2] decision-making based only on biological effects testing, [3] decision-making based on a combination of 1 and 2.

The third option was recommended for Hong Kong because it is the most reasonable for several reasons. First, the use of sediment quality values enables immediate assessment of the likelihood for adverse biological effects. Second, biological testing methods are not well developed for subtropical Hong Kong and their development is expected to take years. During this development period dredging and disposal will still occur, requiring decision-making. However, Hong Kong sediments can and have been tested using established 'international' sediment toxicity tests. Third, neither the use of numbers nor of biological testing are perfect, but together these two approaches provide the most reasonable information for decision-making.

As previously noted, Hong Kong sediment quality values needed to be rationalized and expanded. In this regard, four separate choices were possible, [1] adopt a particular set of 'international' numbers (e.g., the co-occurrence approach, Long et al., 1995); the apparent effects threshold (AET) approach (U.S. EPA, 1996); the equilibrium partitioning (EqP) approach (DiToro et al., 1991), [2] determine some composite approach using 'international' numbers' [3] develop site-specific numbers through field and/or laboratory testing, [4] combine 1 or 2 with 3. We chose the fourth option. Specifically, we developed a composite of 'international' numbers maximizing consistency with the present Hong Kong sediment quality 'criteria', and recommended a process for refining these numbers based on site-specific information (e.g., biological effects testing).

Perhaps even more importantly, we called our numbers 'interim sediment quality values' (ISQV). Two sets of values were recommended for decision-making [1] below which adverse biological effects are unlikely (small type I error: ISQV-low), and [2] above which severe adverse biological effects are very likely (small type II error: ISQV-high). Using two sets of sediment quality values provides three different categories of sediment based on chemical screening: uncontaminated, moderately contaminated, and highly contaminated.

We based our ISQV on the co-occurrence values of Long et al. (1995), which provide two sets of values similar to what we required (but see below), specifically Effects Range Low (ERL) and Effects Range Median (ERM). The AET approach only provides a single value; subjective safety factors are needed to derive more than one value per contaminant (U.S. EPA, 1996). Long et al.'s (1995) values show a reasonable relationship in most cases for concentrations and effects (Ingersoll et al., 1966; MacDonald et al., 1996), despite criticisms of this approach (e.g., Sampson et al., 1996a,b). Moreover, in contrast to most effects data bases which are restricted to temperate regions, they include some data from tropical areas which are applicable to Hong Kong. They also provide data for both metals and organics (the EqP approach is presently only applicable to non-polar organic chemicals and proposed sediment quality criteria only exist for five such chemicals - U.S. EPA, 1996).

Selection of appropriate low-end and high-end ISQV values was reviewed in the context of the current Hong Kong contaminated sediment disposal strategy. The ERL values provide a basis for identifying dredged sediment with contaminant concentrations rarely associated with adverse biological effects and which are therefore suitable for open ocean disposal. The use of ERL values in Hong Kong is appropriate given that adjustments can be made to reflect local conditions. ERM values, however, simply define contaminant concentrations which are frequently associated with adverse biological effects. Thus, dredged sediment with contaminant concentrations above ERM values will include a range of materials which are either suitable for confined marine disposal or require non-marine disposal. This is in contrast with the objective of the current Hong Kong contaminated sediment disposal strategy to require non-marine disposal for dredged sediments which are so toxic (i.e., severe adverse biological effects) that even very small losses to the environment during confined marine disposal are unacceptable. Therefore, ERM values were proposed as working numbers only (i.e., not for decision-making).

ISQV values were not adopted for contaminants just because they exist; for a contaminant to be considered for ISQV development, it had to meet prioritization criteria consistent with international initiatives and regulations regarding priority substances, environmental fate and effects characteristics, and be relevant to Hong Kong. There were cases in which the Long et al. (1995) values were not appropriate either because they did not provide values for a particular contaminant, or because there was a poor concordance with the incidence of adverse biological effects. In such cases ISQVs were not set because screening values are liable to misuse even though they can be reliable interpretive tools.

The end result was [1] inclusion of values for arsenic, total and individual PAH, total PCBs, and total DDT, [2] changes to existing metals 'criteria', [3] replacement of the 'criteria' designation with 'interim sediment quality values' (ISQV), in recognition of current deficiencies in the knowledge base, and [4] two ISQV values (ISQV-low and ISQV-high) for chemical screening, allowing for three categories of sediment: uncontaminated, moderately contaminated, and highly contaminated.

It is recognized that ISQV values are not ideal since they are primarily based on temperate data. Although they do not appear to be inappropriate in comparison to the range of chemical concentrations determined to date in Hong Kong sediments, site-specific data are needed to confirm these values or revise them.

ISQVs can be used for three purposes, [1] identifying and delineating areas of concern, [2] as a basis for identifying site and/or situation-specific contaminants of concern, and [3] as part of a weight-of-evidence approach for decision-making. Limitations to ISQVs include the fact that they [1] do not consider synergism between chemicals, [2] are based on biological receptors only and do not consider human health, and [3] do not consider biomagnification or secondary poisoning. Note that the above uses and limitations to ISQVs also apply to other sediment quality values (e.g., approaches and values summarized in U.S. EPA, 1996).

For the specific purposes of dredging, we recommended a decision-tree for use of ISQV. The first step of this decision-tree would be compilation of existing information to determine whether the sediment is or is not a carrier of contaminants. If it is not, open marine disposal is appropriate. If it is a carrier of contaminants, then ISQVs are used as part of chemical screening to separate the sediment into three different categories. Uncontaminated sediment would have contaminant concentrations below the ISQV-low values, for which biological effects would be unlikely. This sediment would be appropriate for open marine disposal. Moderately contaminated sediment would have contaminant concentrations above the ISQV-low values, for which biological effects are likely. This sediment would require biological screening, with demonstration that lethal and sublethal effects are not of concern before open marine disposal was permitted. Alternatives if adverse biological effects were determined include confined aquatic disposal or, for sediment unsuitable for this alternative, non-marine disposal. Highly

contaminated sediment would contain contaminant concentrations above the ISQV-high, and for which severe biological effects are very likely. In this case the sediment would be considered unsuitable for marine disposal unless the proponent proved otherwise through biological assessment (e.g., tailored bioassays, benthic community assessment, risk assessment).

Refining ISQVs, including formal determination of ISQV-high, will require synoptic chemical and biological effects data. Such should involve: region-specific biological testing (bioassays) and observation (resident community composition) relative to contaminated sediments; specific experimentation (e.g., spiked sediments); data from all appropriate monitoring programs; retrospective ecological analysis of existing benthic community structure related to sediment contaminant concentrations. ISQVs should also be updated based on appropriate new information (e.g., changes to 'international' sediment quality values). A framework needs to be developed for data integration, specifically adding contaminants, refining values, and dropping the 'interim' designation for ISQVs. Upgrading ISQVs will occur based on use and experience (i.e., the answer to the question "Are they suitable and reasonable?"). Validation requires site-specific toxicity testing, field studies, and risk assessment. Their incorporation into a regulatory framework requires consideration of the desired level of flexibility; for instance, 'criteria' are much less flexible than the presently recommended 'values'.

In conclusion, a balance has been attempted between the ideal (biological effects testing) and the reality (decisions needed now). The uses of ISQVs have been clearly proscribed - they should be used for screening only, with biological effects data needed for final decision-making in the case of contaminated sediments. Perhaps most importantly, the impetus has been provided for development and application of sediment biological effects testing in the Hong Kong region by including confirmatory biological testing in dredged sediment evaluations. Overall, this work provides the basis for developing region-specific biological effects data.

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THE SCIENTIFIC AND REGULATORY CHALLENGE OF INTERPRETING CHRONIC SUBLETHAL SEDIMENT BIOASSAYS

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The most commonly used approach for assessing the potential toxicity of sediments involves short-term bioassays which use lethality as an endpoint. Chronic sediment bioassays are being developed and used to evaluate effects of longer-term exposures to relatively low contaminant levels. Interpreting such tests represents a formidable challenge considering the subtle nature of the effects being measured. Sublethal endpoints (e.g., growth and reproduction) will respond not only to the presence of contaminants, but to a number of other environmental factors (e.g., grain size distribution, concentration of suspended sediment) that produce so-called non-treatment effects. In addition, evaluating the ecological relevance of small changes in growth and reproduction is problematic. To meet this specific scientific and regulatory challenge we have developed population models for the test organisms we use in chronic sublethal bioassays (e.g., *Neanthes arenaceodentata, Daphnia magna*). Matrix population models were constructed for these organisms to evaluate the importance to population dynamics of relatively small treatment induced changes in survival, growth, and reproduction. Population models can provide the needed ecological foundation for interpreting chronic bioassay results by linking effects observed on individuals during a bioassay to potential effects on populations in nature.

TEST OF SEDIMENT QUALITY NEAR AN OCEAN DOMESTIC OUTFALL SEWER ON THE POLYCHAETOUS ANNELID *Neanthes arenaceodentata*

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Screened sediments taken from the vicinity of the Orange County, CA Sanitation District outfall sewer were tested for toxicity, on the polychaete *Neanthes arenaceodentata*. Endpoints were survival, growth (dry weight), reproduction and bioaccumulation. Experiments for survival and growth utilized 2-3 wk post-emergent juveniles and subjected them for a 20 d period to sediment taken from increasing distance from the point of discharge. Controls were sea water without sediment, inert sediment and reference sediment. Animals were fed during the experiment. No differences were noted in the survival of animals in any of the treatments, reduced growth was observed in the sea water and inert sediment controls but not in any of the sediments from the discharge area indicating that the worms were obtaining some nutrients from the sediment. In the reproductive and bioaccumulation tests were exposed to sediment for 35-40 d at which time some of the worms were paired for the reproductive test and the remaining worms were frozen after depuration to measure for bioaccumulation contaminants. No differences were noted in the number of young produced in any of the sediment exposures. Differences in the uptake of some metals occurred, but they were not significant not did they impair the biology of the worms.

ENVIRONMENTAL OCCURRENCE AND TOXICITY OF FLUORIDE IN SEDIMENT NEAR A PHOSPHATE FERTILIZER PRODUCTION PLANT IN NORTHERN NEW BRUNSWICK

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The Belledune Fertilizer Plant, Belledune, NB, was recently identified as the largest Canadian discharger of fluoride in the aquatic environment (4,000 tonnes per year) by the Priority Substances List Assessment Program, Canadian Environmental Protection Act. The final effluent from that plant, and sediments in the Baie du Chaleur adjacent to the plant, were chemically characterized for fluoride concentrations. Mean fluoride concentrations in the solids portion of the effluent were 3,730 mg/kg (dry wt.) and were six times higher than dissolved fluoride concentrations in the effluent. Fluoride concentrations in sediments were significantly higher at four sampling stations 100 and 200 m from the outfall of the plant (3,730 to 4,060 mg/kg) compared to other sampling locations. Those concentrations were one order of magnitude higher than concentrations measured at sampling stations 500 m from the outfall and two orders of magnitude higher than those from two reference stations 15 and 22 km from the facility. In addition, sediments adjacent to the plant were evaluated for acute and sub-lethal toxicity. Sediments collected at the four stations with the highest fluoride concentrations were highly toxic to the marine amphipod *Amphiporiea virginiana* (1 to 30% survival) and echinoid *Lytechinus pictus* (<1% fertilization in undiluted porewater, IC_{50} 5.0 to 5.21%). Porewaters tested using the marine bacterium *Vibrio fischeri* were toxic at those stations (15 min IC_{50} 22.1 to 47.4%) however sediments from those stations were not toxic to *V. fischeri* using the solid phase Microtox® test. Sediments and porewaters tested from all other stations and reference sites were not toxic or less toxic to the organisms tested.

INFLUENCE OF AMMONIA ON SEA URCHIN POREWATER TOXICITY TESTS

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The concentration of ammonia in sediment pore water is commonly elevated above ambient overlying water concentrations. The porewater ammonia concentrations are controlled by a myriad of factors including sediment grain size, TOC, temperature, and the microbial, meiobenthic, and macrobenthic communities. The concentration of unionized ammonia in pore water has been observed at levels above which toxic responses are known to occur in sensitive toxicity tests such as the embryological development and fertilization tests with sea urchins. Highly significant statistical associations have been observed between unionized ammonia concentrations in pore water and toxicity measured with the sea urchin assays even though the majority of the samples had unionized ammonia concentrations below the LOEC. Using data compiled from numerous recent sediment quality assessment surveys along the Atlantic and Gulf coasts of the US, the relationship between unionized ammonia, toxicity, and sediment contaminant concentrations was explored and threshold and probable effects limits for unionized ammonia concentrations in pore water were calculated for the sea urchin assays. The correlation analysis revealed that there were highly significant associations between unionized ammonia concentrations and a variety of metals, PAHs, and chlorinated organics for both sea urchin assays. This result suggests that the significant associations observed between unionized ammonia in pore water and toxicity are covarying with the contaminant concentrations. When contaminants are present in toxic concentrations benthic communities can be deleteriously impacted resulting in decreased bioturbation which results in elevated ammonia concentrations.

IDENTIFICATION OF THE METAL RESPONSIBLE FOR TOXICITY IN SEDIMENTS CONTAMINATED BY MINING ACTIVITY, USING TOXICITY: ACCUMULATION RELATIONSHIPS IN *Hyalella azteca*

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The concentration of metals in sediments and water are not sufficient to implicate a metal as the causative agent of toxicity. Metal concentrations in the environment are unreliable indicators of effects because toxicity is a function of metal availability, which is controlled by chemical speciation, adsorption, and interactions between the organism, contaminant, and environmental parameters. The concentrations of Cu and Zn in sediments from Manitouwadge Lake, ON, did not correlate with short term (1 wk) or chronic (4 wk) sediment toxicity. Toxicity did, however, correlate with bioaccumulation of Zn, but not Cu. Furthermore, the close similarity between toxicity:accumulation relationships from Manitouwadge Lake and those obtained from Zn-spiked Hamilton Harbour sediments indicate that toxicity is due to Zn itself, and not some other chemical which correlates with Zn in sediments. Unlike chronic Zn toxicity, and 1 wk Cu toxicity, the chronic toxicity of Cu cannot be predicted from bioaccumulation because bioaccumulation is not significantly elevated at the lower Cu concentrations causing chronic toxicity in spiked sediments. Growth, however, is reduced by Cu, and not by Zn. The absence of a significant growth reduction in Manitouwadge Lake sediments strongly suggests that the contribution of Cu to chronic toxicity was minimal. Short term (1 wk) metal accumulation appears to be a reliable means of identifying short term Cu and Zn toxicity, and chronic Zn toxicity; identification of chronic Cu toxicity requires additional data, such as growth impairment.

TOWARD A FIELD ANALYTICAL PROCEDURE FOR DETERMINING ACID-VOLATILE SULFIDE USING AIR

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A purge-and-trap procedure using air for the carrier gas, and 50 ml centrifuge tubes for the reaction vessels, is described for determining acid-volatile sulfide (AVS) in sediment. The use of air for the carrier gas did not result in significant sulfide oxidation. Sulfide concentrations in eight sediment samples containing AVS (i.e., particulate FeS) measured using the air-AVS method were not statistically different ($\alpha = 0.05$) than concentrations measured using the standard, nitrogen-based AVS method. Recovery of aqueous sodium sulfide spikes, ranging from 10.2 to 42.4 μmol added, was 91.67±6.98% (N = 20), using both vacuum and positive-pressure air flow. Kinetics experiments showed that sulfide transfer from acidified sodium sulfide spikes in the small-scale analytical train was complete in approximately 10 min. Sulfide transfer from acidified sediment samples may have reached completion in about 30 to 40 min. An essential feature of the new method is the inclusion of an acid reservoir tube in the analytical train. Acid transfer to the sample, and H_2S transfer to the sulfide trap solution, were simultaneously initiated by the air flow. If adapted for use in the field, the method may be well-suited for providing screening-quality AVS data from a large number of samples (e.g., over a large spatial area at a site) rapidly, safely, and economically.

TWO-STAGE EXPLORATORY ECOTOXICOLOGICAL HAZARD EVALUATION OF A CONTAMINATED SITE IN THE SOREL DELTA AREA

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This presentation will focus on the design and results of a two-stage exploratory hazard evaluation conducted on a contaminated site located on the shore of Ile aux Corbeaux (Sorel, QC). Used batteries, which originally served as energy sources for navigation beacons, were found buried in several sites on islands of the Sorel delta (St. Lawrence River). At the study site, soils and sediments were found to be contaminated with heavy metals which probably originated from these batteries. The first stage consisted of a physico-chemical and bioanalytical characterization (i.e. mainly granulometry, heavy metal content and acute sublethal toxicity using a bacterial solid-phase test) of 34 substrate samples. The second stage concerted efforts on six target composite samples and broadened effect evaluation to include [1] mortality of and heavy metal bioaccumulation in earthworms, [2] germination and growth of cress, and [3] growth and an innovative solid-phase test on phytoplankton. The main conclusions of the study, which served to establish Environment Canada's position relative to the management needs of the study site, will be presented.

TOXICOLOGICAL CHARACTERIZATION OF LAKE CHAPALA

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Toxicological analyses of organic and inorganic extracts of sediments taken from lake Chapala were carried out. Sediment samples were collected every three months for one year. Heavy metals were monitored and a preliminary characterizations of selected toxic extracts were made. Organic extracts were evaluated using the *Photobacterium phosphoreum* acute toxicity test (Microtox®). Heavy metals concentrations were determined by atomic absorption. The compounds in the organic extract were separated, isolated by chromatography and later characterized. Apparent changes in toxicity distribution were detected over the four sampling seasons. That could not be related to heavy metals which has homogeneous spatial and temporal distributions and similar concentration levels to unaffected aquatic systems. The preliminary analyses by gas chromatography of the organic extracts showed the presence of some compounds widely distributed in the lake. The origin of such compounds may be related with the Lerma River inlet. Other abundant and poorly distributed compounds were also found, associated with local pollution sources. The identification of potentially toxic organic compounds is underway using preparative chromatography followed of conventional spectroscopy techniques.

COMPARISON BETWEEN MICRO-SCALE AND CONVENTIONAL WHOLE SEDIMENT ASSAYS FOR FRESHWATER SEDIMENT TOXICITY ASSESSMENT

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The use of several micro-scale assays for evaluating freshwater sediment toxicity was investigated to develop a representative and cost-effective test battery. The bioassays evaluated (18 assays total) included micro-scale assays performed on solid phase, pore water and organic extracts as well as conventional standardized sediment assays with *Chironomus riparius* and *Hyalella azteca* on ten sediment samples collected in the St. Lawrence River/Great Lakes systems. Selected sediment physical and chemical characteristics were also conducted to aid in the interpretation of results. *C. riparius* and *H. azteca* endpoints were found to correlate well to contaminant levels. Assays such as ATP (adenosine tri-phosphate) measurement of sediment microbial biomass gave promising results to predict whole-sediment toxicity to benthic invertebrates. Most assays on pore water did not adequately

represent whole-sediment toxicity while organic extracts appeared to be more representative, alleviating some potential confounding factors such as interference due to toxicity caused by ammonia.

SOIL ECOTOXICOLOGY
SESSION CHAIR: G. SUNAHARA

THE USE OF *IN SITU* BIOASSAYS FOR SOIL INVERTEBRATES: WHAT DO THEY REALLY TELL US?

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At present, any effects assessment of chemicals for the terrestrial ecosystem is based on very few soil toxicity data. For this reason, often ecotoxicity data for aquatic organisms are used, applying the equilibrium partitioning theory, to derive safe levels of chemicals in the soil. To relieve the dependence on aquatic toxicity several truly terrestrial tests are at various stages of development. However, it is necessary that any test allows for an ecologically realistic effects assessment. In addition to these tests, several modified bioassay techniques have also been developed from traditional aquatic assays. It is envisaged that such tests may be useful in the determination of the potential risk of contaminated soils or the quality of decontaminated soils. It is also considered that such bioassays are higher stage tests for the overall effects assessment procedure. The results of several trials with various bioassays in Africa and the UK, will be presented and their advantages and limitations discussed. Do they tell us what we really want to know?

TOXICITY TESTING APPLIED TO SOIL ECOTOXICOLOGY - REPORT OF THE FIRST NRC WORKSHOP PROCEEDINGS

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The first NRC workshop on Ecotoxicity was organized in November, 1995, to discuss and develop a Canadian position on the problems related to the toxicity testing applied to contaminated soils. Two tasks were identified for the workshop, [1] methodological issues in toxicity testing of contaminated soils, and [2] application of toxicity tests in contaminated sites management. A series of technical presentations and workshop sessions drew three major conclusions. First, it was noted that existing documentation on terrestrial toxicity testing does not clearly address issues concerning sampling requirements, soil preparation and toxicity results relevance. Those limitations tend to cause confusion on the potential uses of terrestrial toxicity tests. Second, it was pointed out that fundamental knowledge on soil matrix and on soil biology is still necessary for one to interpret with more confidence the potential effects on the target organisms. Comparative studies with different types of soil, bioavailability of contaminants in soil and soil ecology are research and development areas that should be promoted. Third, there is a necessity to establish a framework allowing the optimal use of terrestrial toxicity tests for contaminated site assessment. Although present protocols are scientifically rigorous, a framework allowing a clear-minded choice of problem-specific bioassays has to be developed. The lack of a clear understanding and consensus on the effects which are ecologically relevant, limits the proper application or interpretation of toxicity data. This workshop has emphasized the necessity to improve the interactions between appliers and users of terrestrial toxicity test data.

DEVELOPMENT OF CCME ENVIRONMENTAL QUALITY GUIDELINES FOR SOILS

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The need for scientifically defensible soil quality guidelines to promote consistent assessment and remediation of contaminated sites was identified as an urgent priority by the Canadian Council of Ministers of the Environment (CCME). In response to this need, the CCME has recently released a national protocol for the derivation of environmental and human health soil quality guidelines to restore and sustain agricultural, residential/parkland, commercial and industrial land uses in Canada. Irrespective of land use, the central guideline concepts are protection of human, plants, and animals from toxic harm, integrity of nutrient and energy cycles, and minimization of cross contamination of the other environmental media. Procedures for deriving environmental soil quality guidelines consider key receptors, exposure pathways, and the level of effects associated with protection of a particular land use for a broad range of activities. For example, an agricultural land use scenario considers the potential risk associated with direct exposure of plants and invertebrates through soil contact and indirect exposure of grazing wildlife and livestock through ingestion of contaminated soil and plants. Final recommendations of environmental soil quality guidelines may also incorporate toxicological information on soil microbial function and may consider the potential risk to aquatic life in associated surface water systems. Currently, the CCME is in the process of developing environmental and human health soil quality guidelines for approximately 20 priority substances based on this protocol. Through illustration of the derivation procedure for several substances, the scope and intent of the guidelines, data limitations, research needs and future directions in the development of soil quality guidelines is highlighted.

THE USE OF TOXICITY ASSESSMENT TO DEVELOP SITE-SPECIFIC REMEDIATION CRITERIA FOR OIL AND GAS FACILITIES.

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A brief guidance manual was produced by Golder and the Canadian Association of Petroleum Producers (CAPP), which is intended for use by those individuals who are familiar with the phased approach to site decommissioning endorsed by the CCME, but who may not be familiar with ecological toxicology and the use of risk assessment to develop site-specific remediation criteria. The manual introduces the basic concepts of toxicity testing, ecotoxicity and risk assessment.

There are two basic approaches for determining remediation criteria for contaminated sites, [1] compare the concentrations of chemicals found on-site with broad regional or national soil and water quality objectives developed for the chemicals of concern, for example, the CCME national guidelines or the Alberta Environmental Protection Tier I criteria (CCME, 1991a; AEP, 1994), or [2] conduct a site-specific risk assessment (i.e., Alberta Tier II approach (AEP, 1994)).

Reliance on the first process may require site cleanup to a level that is overly protective and cost prohibitive. General criteria tend to be conservative, do not take into account site-specific factors that affect the bioavailability of contaminants, and do not account for potential interactions among the components of complex mixtures of contaminants. Alternatively, because established criteria do not exist for all chemicals and stressors, it is also possible for concentration-based assessments to underestimate the potential threat a site poses to the environment.

Risk assessment is a site-specific process that results in remediation objectives that satisfy the true intent of remediation, that is, to remove all impairments to the natural ecological capacity of the site and

restore the site to a level consistent with the expected end use. Toxicity tests are used to expose test organisms directly to the media (soil, water or sediment) suspected of being contaminated, and to evaluate the effects of any contamination on the survival, growth, reproduction, behavior and/or other attributes of the test organisms. While not directly relevant to assessments of human health, ecological toxicity or 'eco-toxicity' testing can be used to develop site-specific, Tier II criteria for the protection of ecological receptors. By directly measuring the integrity of biological organisms, toxicity tests can be used to [1] alleviate problems associated with overly conservative chemical criteria, [2] alleviate problems associated with incomplete chemical profiles, [3] evaluate the aggregate toxic effects of all chemicals in a mixture, [4] demonstrate whether chemicals are present in a form which is biologically available for uptake, [5] evaluate the toxicity of substances whose biological effects are not well known, [6] characterize the distribution of toxicity at a site, [7] develop site-specific remedial goals, [8] assist in site monitoring during and after remediation, and [9] assist in determining a site's post-remediation potential to support a viable ecological community (e.g., agricultural sustainability of the site).

This guidance manual is the end result of a two year study into the evaluation of toxicity-based methods to develop site-specific, risk-based cleanup objectives for the decommissioning of oil and gas facilities (EMA, 1994, Hamilton et al., 1994; Golder Associates Ltd., 1995). The proposed approach involves using a battery of eco-toxicological assays at various stages of the phased decommissioning process endorsed by the CCME (CCME, 1991b) to directly quantify the hazards to organisms at all trophic levels and in both terrestrial and aquatic ecosystems from chemical residues at contaminated sites.

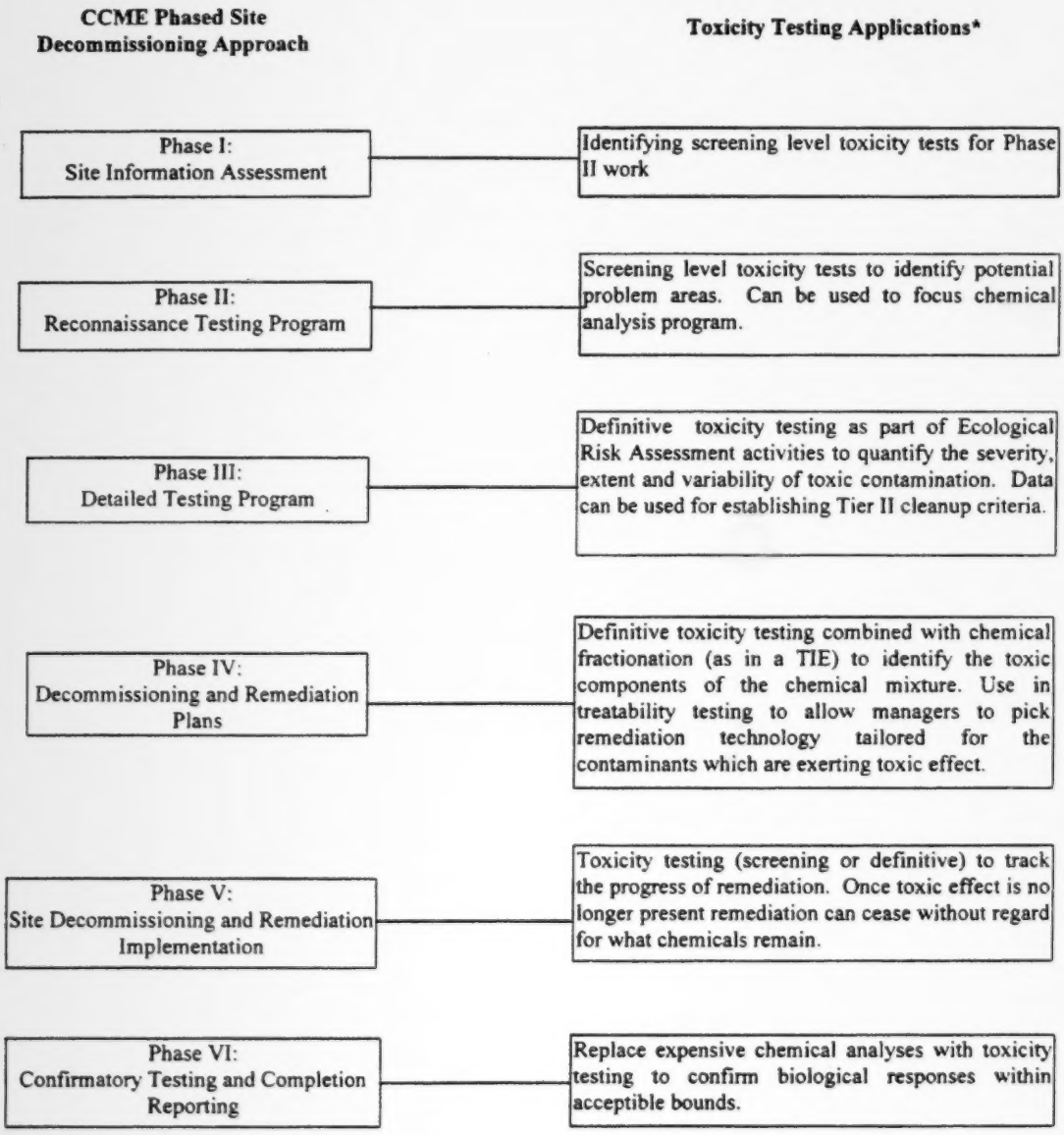
The National Guidelines for the Decommissioning of Industrial Sites, prepared by CCME (1991b) and adopted by Alberta Environment, rely on a phased approach for determining if a potentially contaminated site poses a threat to human health or the environment, and the level of remediation required (Fig. 1). Subsequent to these guidelines, the Alberta and British Columbia provincial environmental departments developed their own chemical-based remediation criteria (e.g., Alberta Tier I), which were more specific to Western Canada and widely used in the Oil and Gas industry for establishing remediation criteria.

Alberta Environmental Protection subscribes to a two-tier approach to setting acceptable concentrations of contaminants in soil (AEP, 1994). Tier I values are generic and approximate acceptable concentrations of soil contaminants for all site conditions and land uses without defining actual risk. In contrast, Tier II criteria are site-specific concerning protection of human health and the environment. Such criteria are based on acceptable risk specific to the site in consideration of such variables as soil, geology, surface and groundwater, climate and land use.

Tier II criteria offer an alternative to the Tier I chemical criteria, and are developed by the proponent. Proponents opting for Tier II remediation criteria must provide credible risk-based scientific documentation in support of their criteria, and be willing to defend them before the public and regulators. Because of the extreme variations in site geology and ecological receptors associated with oil and gas operations, more site-specific assessment and remediation criteria (Tier II) should be developed. Fig. 1 shows the applications of toxicity assessments in developing site-specific remediation objectives within existing CCME industrial site decommissioning process. Identification of problem areas using screening level toxicity tests during Phase II can focus chemical analyses on just those samples known to be toxic, thus avoiding expensive screening level chemical analyses on a large number of non-toxic samples.

FIGURE 1

THE USES OF TOXICITY ASSESSMENT WITHIN THE EXISTING SITE DECOMMISSIONING, REGULATORY FRAMEWORK


CCME Phased Site Decommissioning Approach
Toxicity Testing Applications*
Phase I: Site Information Assessment
Identifying screening level toxicity tests for Phase II work
Phase II: Reconnaissance Testing Program
Screening level toxicity tests to identify potential problem areas. Can be used to focus chemical analysis program.
Phase III: Detailed Testing Program
Definitive toxicity testing as part of Ecological Risk Assessment activities to quantify the severity, extent and variability of toxic contamination. Data can be used for establishing Tier II cleanup criteria.
Phase IV: Decommissioning and Remediation Plans
Definitive toxicity testing combined with chemical fractionation (as in a TIE) to identify the toxic components of the chemical mixture. Use in treatability testing to allow managers to pick remediation technology tailored for the contaminants which are exerting toxic effect.
Phase V: Site Decommissioning and Remediation Implementation
Toxicity testing (screening or definitive) to track the progress of remediation. Once toxic effect is no longer present remediation can cease without regard for what chemicals remain.
Phase VI: Confirmatory Testing and Completion Reporting
Replace expensive chemical analyses with toxicity testing to confirm biological responses within acceptible bounds.

* NOTE: Obtain regulator approval of proposed toxicity screening program(s).

During Phase III, definitive toxicity tests coupled with focused chemical analyses can be used as part of risk assessment activities to quantify the extent and degree of the contamination. Once chemical characterization and definitive toxicity testing has been completed, remediation planning can begin by back-calculating to non-toxic chemical concentrations (remediation objectives) using dilution series toxicity test results. For example, for agricultural or pasture land end use, site-specific grass or crop seed emergence tests can be used to confirm that re-vegetation will occur following a given level of remediation at the site.

If a more detailed characterization of the toxic component in a chemical mixture is desired, (i.e., to assist in selection of remediation technologies) Phase IV toxicity tests can be combined with systematic chemical fractionation of the soil and water samples. This is accomplished through a Toxicity Identification and Evaluation (TIE) to identify the portions of a complex mixture that are exerting the toxic effect, focusing detailed chemical analyses on only those compounds that are known to be toxic. This knowledge of the toxic contaminants helps project managers to pick a remediation plan that is specifically tailored for both the class of chemicals exerting the toxic effect and conditions specific to that site.

During Phase V activities, toxicity testing can be used to track the progress of remediation activities. Once no further toxicity can be demonstrated, active remediation efforts can confidently cease without regard for what chemicals remain.

Confirmatory chemical analyses in Phase VI can also be replaced by toxicity testing to confirm that biological responses at the remediated site are within acceptable, targeted levels. For example, toxicity tests using aquatic organisms can be used to confirm that remedial activities have restored a river or lake to the level necessary to support appropriate aquatic life.

The manual provides guidance on performing a toxicity assessment of a site, including site soil sampling, toxicity test battery selection, choosing a toxicity testing laboratory and interpreting toxicity data. A battery of several toxicity tests, including a suite of test species representing different phyla and trophic levels, are strongly recommended for contaminated site assessment. Test batteries provide information on the variety of organisms that may be at risk from contaminants at the site and allow for the possibility that different contaminants may be affecting different species. Because of the varied responses of different species to contaminants, at least three species should be included in the test battery. The test batteries recommended by Keddy et al. (1993) and Environment Canada (1994a) for freshwater, sediments and soils are presented as an appendix in the manual.

The actual test battery should reflect the site-specific environment and end use of the site following its decommissioning. The selected toxicity test battery should be agreed to by the appropriate regulatory body for the site in question, prior to starting the toxicity assessment study.

The Environmental Risk Assessment paradigm for contaminated sites is also described (Environment Canada, 1994b) consisting of the four steps, [1] receptor characterization, [2] hazard assessment, [3] exposure assessment, and [4] risk characterization. The ultimate goals of ERA, in the context of site remediation and decommissioning, are (Environment Canada, 1994b) [1] to estimate the extent and nature of risk to the ecosystem from the contaminants, and [2] to determine the most effective strategy for remediation through the establishment of site specific remediation goals or objectives.

Another method for defining site-specific remediation criteria is described, which has been recommended for use in the decommissioning of oil and gas sites in Canada is the Toxicity Identification and Evaluation (TIE) procedure (Hamilton et al., 1994; Golder Associates Ltd., 1995). This approach uses a coordinated sequence of chemical analyses and toxicity testing designed to isolate and rank the dominant toxic fractions in complex chemical mixtures. A typical use of toxicity testing with a treatability test is described below for the example of decommissioning a gas plant flare pit. If the pit material

contains significant weathered oil containing polynuclear aromatic hydrocarbons (PAH) and brine contamination, conventional knowledge suggests that a certain amount of salt and oil must be removed from the soil to allow proper re-vegetation of the site. While chemical criteria exist that determine a reduction in electrical conductivity to below 2 dS/m, total non-chlorinated PAH below 1 mg/kg and total mineral oil below 1000 mg/kg, it may be very difficult to rapidly achieve these cleanup criteria in a simple on-site bioremediation approach, unless significant contaminated soil dilution is possible. However, by establishing the initial soil toxicity to plants and earthworms (i.e., LC_{50}), an indication of how clean the soil needs to be to adequately eliminate the toxicity of this site-specific ecosystem is obtained. A TIE could then be used to determine which contaminant is causing the residual toxicity.

The treatability test would then be designed to determine if the new toxicity-based cleanup criteria can be achieved. A successful treatability test of a suitable desalination technology and bioremediation of the hydrocarbons to site-specific cleanup criteria, back-calculated from the LC_{50} data, can then be followed by final toxicity testing to confirm the removal of toxicity to the test battery. The final cleanup criteria may be significantly higher than the Tier I criteria, but will have been shown to be non-toxic under the site-specific conditions. These treatability test results can then be used by the proponent to support an application for site remediation and subsequent reclamation certificate from the provincial environmental authority. Once the decision to proceed with remediation has been made, toxicity testing, coupled with site-specific assessment techniques, can be used to plan, direct and monitor the progress of remediation.

To summarize the approach outlined in the manual, the risk assessment procedure, through hazard and exposure assessment, provides for site-specific quantification of the risks associated with complex chemical mixtures present in equally complex ecosystems. During remediation activities, risk can be monitored throughout the process until it is within acceptible bounds. Using the TIE approach, the dominant toxic fraction of a complex chemical mixture can be identified, and detailed chemical analyses can then focus on just those fractions which are found to exert toxic effects, thereby reducing significant analytical expenses. Also, by identifying the dominant toxic components of a mixture during a TIE, a remediation strategy can be designed to focus on the classes of contaminants identified. Treatability tests can be used to assess the ability of various remediation technology options to effectively reduce toxicity to target organisms.

In addition to providing clear and unambiguous targets for site cleanup and providing data to assist with remediation technology selection, toxicological endpoints can also be used to monitor the progress of remediation measures as they are applied. Once no further toxicity can be demonstrated, remediation can confidently cease without regard for what chemicals remain.

To obtain a copy of the manual, please contact the Canadian Association of Petroleum Producers, 2100, 350 - 7th Avenue S.W., Calgary, AB, T2P 3N9.

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ECOTOXICITY TESTING AS A MONITORING TOOL FOR BIOREMEDIATION OF HYDROCARBON-CONTAMINATED MATERIALS

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Ecotoxicity tests, respiration rates and hydrocarbon content were used to monitor the bioremediation of a crude oil spill agricultural soil, a diesel invert mud (DIMR) and a flare pit waste treated for varying lengths of time in a Bio-Reactor and Bio-Pile (Bio-Reactor Project). Following aggregation and leaching with water to reduce the $EC_{1:2}$ from over 25 dS/m to <4.0 dS/m, each waste was fertilized, cultivated, heated and irrigated to optimize conditions for biological activity. In the agricultural soil, the hydrocarbon content decreased from 4.3 to 2.2% during 16 mon bioremediation, in the DIMR it fell from 10.8 to <0.7% after 21 mon treatment, while in the flare pit waste it was reduced from 8.5% to an estimated 3% following 10 mon treatment (A. Kharrat, pers. comm.). Disappearance of the hydrocarbon was accompanied by a reduction in soil/waste respiration rate from 6.8 to 0.2 :g CO_2-C/g/h in the agricultural soil, from 6.0 to 0.6 :g CO_2-C/g/h in the DIMR and from 3.3 to 0.4 :g/g/h in the flare pit waste.

Ecotoxicity was monitored using a series of acute (single species) bioassays including earthworm survival in bulk soil/waste, germination and root elongation by lettuce, canola and barley in bulk soil/waste; and Microtox® on a 1:4 soil:water extract. Both the DIMR and flare pit waste were extremely toxic initially but after 10 mon treatment the flare pit waste was no longer toxic. Following 21 mon of bioremediation, the DIMR was nontoxic to earthworms, but still retained some phytotoxicity. No toxicity was found in the agricultural soil after 16 mon bioremediation. Treatment and ecotoxicity endpoints did

not coincide and toxicity could not be predicted from the hydrocarbon content. Although the agricultural soil and flare pit waste achieved treatment and toxicity endpoints, residual hydrocarbon contents exceeded current regulatory guidelines.

EFFECTS OF SIMULATED ACID RAIN ON BELOW-GROUND COMPONENTS OF AGRICULTURAL CROPPING SYSTEMS ON WESTHAM ISLAND, BRITISH COLUMBIA

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OBJECTIVES

The overall aim of the ecotoxicological field study was to evaluate possible effects of simulated acid rain on nutrient and heavy metal budgets in red clover and pea cropping systems. Earthworms and other soil animals were used as bioindicators of possible changes in soil biological components of the cropping systems. The effects of the treatments on the biomasses and elemental budgets of key soil animals were determined to evaluate toxicological effects on the bioavailability of major nutrients (other than nitrogen) and heavy metals in the treated plots.

METHODS

Westham Island is approximately 40 km south of Vancouver, BC. The project took place between mid November, 1982, and late August, 1984. The soil was a silty clay loam derived from deltaic deposits. A 3 x 3 Latin Square was set up in a 14 mon old red clover field. Each plot consisted of nine 2 m x 2 m subplots with buffer strips of one and four meters between subplots in each plot and between plots, respectively. Acidified water was sprayed on the plots every two weeks. Sufficient hydrogen ions using a 1 N solution of Sulfuric and Nitric acids (5:1 eq. Ratio), were applied to lower the over all monthly pH of precipitation to 4.3 and 4.0 in treatments A and B respectively and 4.8 in control plots. The latter pH is the average pH of the rain in lower mainland British Columbia.

Only the control and B treatments plots were quantitatively sampled although soil pH and available soil pools of elements were measured in plots of both treatment and in controls. Above and below-ground parts of plants and soil were sampled quantitatively at intervals before and during the acidification experiment. The sample size for earthworms, macro-arthropods and plant roots was 0.063 m^2 x 0.15 m deep. For above-ground parts of plants, quadrat frames of 2 x 1 m^2 were used. Soil for pH and chemical analyses was collected with a corer of 0.00229 m^2. Individual earthworms and beetles were washed with distilled water and their guts voided. For chemical analyses, samples were digested with Nitric Acid. Digests were analyzed using a AAS spectrophotometer whilst Cd analyses were done using a HGA-2100 graphite furnace. U.S. National Bureau of Standards orchard leaves and bovine liver, standard soils and an internal worm standard were analyzed with each batch of samples.

RESULTS

Soil Fauna: No effects of the treatments on populations of soil millipedes, centipedes and staphylinid adult beetles were observed between November, 1982, and March, 1983. Carabid larvae (*Pterostichus melenarius*) were of significantly lower biomass in the acidified plots in March 1983 but this pattern was not observed with the next generation of larvae sampled in March, 1984. By March, 1983, population biomass of earthworms in both control and treated plots had decreased from the peak observed in the 1982 autumn prior to the acid rain experiment. Biomass was very low after the old red clover crop was ploughed under in April, 1983. In mid December, total earthworm biomass had increased substantially and was lower in the acidified plots but not significantly so. However, the major species, *Lumbricus rubellus* was affected by the acid rain (Table 1).

Table 1. Standing crops of surface litter (g/d/wt/m^2), soil pH and population biomasses (g/d/wt/m^2) of two earthworm species (g/d/wt/m^2), in control and treatment plots of simulated acid rain experiment. Standard deviation shown in brackets. pH of mineral soil (0-10 cm) measured in 0.01 M $CaCl_2$ in 1:2.

	Before Expt.	During Experiment			
	Nov. 15, 1983	Mar. 15, 1983	June 5, 1986	Aug. 19, 1983	Dec. 11, 1983
CONTROL PLOTS					
Litter	514.499 (411.950)	263.600 (63.730)	0.000	ND	76.700
Soil pH	5.500 (0.480)	5.200 (0.280)	4.900	4.800 (0.690)	5.000 (0.460)
L. rubellus	22.688 (14.872)	10.838 (8.989)	0.519	5.348 (2.743)	23.293 (11.310)
A. chlorotica	5.376 (1.740)	4.791 (3.581)	0.658	1.897 (1.690)	2.526 (2.388)
ACIDIFIED PLOTS					
Litter	464.88	336.200 (82.900)	0.000	ND	89.100
Soil pH	ND	5.600 (0.860)	5.200	4.900 (0.440)	5.200 (0.750)
L. rubellus	18.2100 (8.1873)	8.254 (4.771)	3.348	3.682 (2.830)	15.222 (7.433)
A. chlorotica	4.5800 (2.4840)	7.486 (4.123)	1.548	2.540 (0.902)	3.239 (2.013)

With the exception of Al for the second earthworm species, *Allolobophora chlorotica*, the treatments did not affect body burdens of elements in the earthworms. There was a significant increase (Wilcoxon 2 sample tests, $p < 0.05$) for Al concentrations in *A. chlorotica* from acidified plots in March and August, 1983.

Elemental Budgets: Budgets for select elements investigated are compared in Table 2. Data for the pea, August, 1983, and red clover cropping systems in December, 1983, are included. In August, 1983, earthworm biomass was low and soil invertebrate predators were rare after the intensive April cultivations. Pea biomass was greater in the acidified plots than in controls, likely because of the 'fertilizer' effects of sulfur in the acidified water.

Earthworms accounted only for a small percentage of the cycling, bioavailable pool of elements, particularly for heavy metals. Nonetheless, the cycling pools of elements such as K (Table 2) and Al and Mn are themselves only a very small proportion of the total system pools and shifts in the cycling pool need to be evaluated.

Between 15 and 22% of the K cycling pool occurred in the above and below ground parts of the plants. This element is considered to be very mobile and is readily lost from plant tissue and polluted acid rain has been found to increase its mobility. In this study, the pool of available soil K, and several other elements, was lower in the acidified plots (Table 2) indicating loss by processes such as leaching. It is important that as much as possible of plant K be conserved and recycled within agricultural cropping systems.

CONCLUSIONS

The study was only a short term one and the data must be interpreted with caution. The changes in relative biomasses of the two earthworm species and in concentrations and amounts of elements in the available soil pools, however, suggest that shifts in major soil components could have occurred if the treatments had continued. The earthworms and available soil pools of elements studied were sensitive indicators of short term changes.

Fully fledged experimental field ecotoxicology studies are costly to carry out but are useful, with well designed laboratory studies, in providing reference data for interpreting changes in the cycling pools of heavy metals and in quantifying the exposure of contaminants to earthworms and other soil invertebrates.

TABLE 2: ELEMENTAL BUDGETS FOR CONTROL AND ACIDIFIED PLOTS IN DECEMBER 1983 AND PARTIAL BUDGET FOR AUGUST 1983

		CONTROL PLOTS							
TOTAL IN VARIOUS POOLS	STANDING CROP OR BIOMASS g d wt m^{-2}	Ca	Mg	Na	K	Fe	Zn	Cu	Mn
Soil (to 10 cm)	115 x 10^3	793.100	1656.100	3096.800	554.600	5472.300	10.940	6.859	56.660
Cycling Pool, red clover system	—	**160.590**	**17.030**	**1.384**	**47.900**	**4.938**	**0.219**	**0.430**	**0.602**
TOTALS IN COMPARTMENTAL POOLS									
PEA SYSTEM, Aug. 1983	582.000	1.925	0.637	2.741	11.211	0.018	0.018	0.199	0.009
Earthworms	7.296	0.026	0.009	0.023	0.059	0.011	0.002	—	—
RED CLOVER SYSTEM, Dec 1983	231.900	0.997	0.387	0.069	5.394	0.099	0.007	0.003	0.005
OTHER VEGETATION									
above ground	72.800	0.315	0.173	0.024	2.024	0.188	0.003	0.001	0.002
roots	320.800	0.056	0.108	0.030	3.189	0.051	0.004	0.002	0.000
surface and soil litter	96.360	0.392	0.456	0.016	0.309	—	0.007	0.004	0.026
SOIL AVAILABLE	—	158.700	15.870	1.150	35.800	4.485	0.150	0.207	0.573
SOIL UNAVAILABLE	—	634.400	1640.200	3095.700	1514.800	5067.800	10.791	6.651	56.085
Earthworms	25.818	0.087	0.0389	0.075	0.191	0.195	0.005	—	—
		TREATMENT PLOTS							
TOTAL IN VARIOUS POOLS	STANDING CROP OR BIOMASS g d wt m^{-2}	Ca	Mg	Na	K	Fe	Zn	Cu	Mn
Soil (to 10 cm)	115 x 10^3	—	1732.67	3752.9	1435.800	5237.200	11.410	6.868	58.480
Cycling Pool, red clover system	—	**238.840**	**18.790**	**1.766**	**40.938**	**4.898**	**0.191**	**0.235**	**0.725**
TOTALS IN COMPARTMENTAL POOLS									
PEA SYSTEM, Aug 1983	778.000	4.352	1.081	0.177	16.445	0.148	0.022	0.001	0.009
Earthworms	6.610	0.022	0.003	0.020	0.051	0.074	0.013	0.014	—
RED CLOVER SYSTEM, Dec 1983	204.700	1.000	0.414	0.044	3.673	0.104	0.005	0.004	0.005
OTHER VEGETATION									
above ground	48.000	0.291	0.147	0.026	1.290	0.269	0.002	0.001	0.008
roots	109.700	0.019	0.037	0.010	1.091	0.001	0.001	0.009	—
surface and soil litter	105.730	0.630	0.343	0.013	0.241	1.010	0.006	0.003	0.019
SOIL AVAILABLE	—	236.900	17.825	1.611	34.500	3.450	0.173	0.219	0.690
SOIL UNAVAILABLE	—	—	1714.146	3251.300	1401.300	5233.800	11.240	6.649	57.790
Earthworms	18.461	0.067	0.021	0.062	0.143	0.044	0.005	—	—

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TOXICITY IDENTIFICATION AND EVALUATION INVESTIGATIONS (TIE)
SESSION CHAIR: D. BIRKHOLZ

USE OF THE TOXICITY-BASED APPROACH IN ENVIRONMENTAL HAZARD ASSESSMENT

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The need to identify the potential hazards from chemical releases into the environment to biological organisms presents an interdisciplinary challenge ranging from toxicology to physical chemistry in which a variety of analytical techniques play a vital role. Hazard assessment problems are compounded when chemicals entering the environment interact with one another, or with the ecosystem. Data required for protection of the environment cannot be obtained from chemical analysis alone. Transformations to which chemicals are subjected after release into the environment are too complex. The toxicity-based approach exposes indicator organisms to substrates containing chemical mixtures that might impact the environment. Biological tests can detect the presence of toxicity and identify the potential for toxic effects on resident species. Since biological organisms are the best indicators of actual or potential disruption of ecosystems a toxicity-based approach should be pursued. Comprehensive hazard assessment requires toxicity data from a variety of species representing several trophic levels. Assessment of 185 environmental samples tested using algae and *Daphnia magna* revealed that testing with *D. Magna* alone would have missed 25% of the toxic samples while testing with algae alone would have missed 15% of the toxic samples. Few hazard assessments, however, include a plant in the suite of indicator organisms. Limitations in both chemical and toxicological analysis requires that a holistic approach be taken in evaluating toxicity. If all the difficulties of using chemical analysis could be disregarded, the fact remains that analysis of chemically complex environmental samples does not answer the fundamental question: Is the substrate under investigation toxic to living organisms?

ISOLATION AND TENTATIVE IDENTIFICATION OF THE COMPOUND RESPONSIBLE FOR FISH MFO-INDUCTION IN BKME

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Pulp mill effluents (BKME) have the potential to cause deleterious effects on aquatic biota in receiving waters. One of the consistent sub-lethal responses has been the induction of mixed function oxygenases (MFO) in fish. Previous research has shown that the MFO (EROD)-inducing chemicals in final pulp mill effluent could be isolated by using C_{18} solid phase extraction. The chromatographic data identified six principal compounds (excluding phthalates). All six compounds were bibenzyl derivatives coupled by either a methane, ethane, or ethene bridge. Of the six compounds, one was a chlorinated stilbene. Comparing data from each of three fractionation trials, EROD activity corresponded best with

the presence of the chlorinated stilbene. The compound is tentatively thought to belong to the pinosylvin family, a group of naturally occurring substances, often present in coniferous trees. Compound identifications were made using capillary column mass spectrometry (GC-MS) with both electron impact and electron negative ion techniques as well as responses from other selective detector systems, such as NPD, FID, ECD, and AED.

ECOLOGICAL IMPACTS OF STORMWATER RUNOFF ON AN URBAN WATERSHED

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The Brunette River watershed, located in the Greater Vancouver Regional District of the Lower Fraser Valley, is highly urbanized and a major transportation corridor through the region. The watershed contains 50% residential and 25% commercial/industrial land use. Traffic density (vehicle km/day) has increased 44% in the last 20 yrs. Intensive monitoring of water quality conditions during baseflow and rainfall events demonstrates that water quality criteria for the protection of aquatic life are exceeded for trace metals, such as Cu and Zn, especially during storm periods. Stream sediments exceed the probable and threshold effects criteria for the trace metals Cu, Hg, Pb and Zn and total petroleum hydrocarbons levels in many areas are high. Although lead in sediments has decreased (35%) in the last 20 yrs due to it's removal from gasoline, Cu, Hg, Mn and Zn have increased by 81, 290, 130, and 45% respectively. Microtox® bioassays demonstrate toxicity in stream sediments and suspended sediments transported during storm events. Chironomid bioassays show sediment toxicity which is highly variable depending upon sedimentation characteristics at the station and the flushing action of previous rainfall events. Best management practices to control sediment transport to the waterways would be a first step to improve the quality of the aquatic environment in this watershed.

ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPONENTS OF LAMPRICIDE (TFM)

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A recent finding has demonstrated that application of pesticides to control the abundance of lamprey in Great Lakes tributaries results in induction of mixed function oxygenase enzymes and disruption of circulating levels of sex steroids in fish. A TIE approach was developed and applied to isolate, characterize and identify the responsible chemicals. Separation of the lampricide components was done using solid phase C_{18} cartridges followed by HPLC and measurement of MFO activity in fish exposed to the selected fractions. The MFO induction results from a trace contaminant in 3-trifluormethyl-*r*-nitrophenol (TFM) with a predicted low Kow of approximately 3.5. A survey of batches from different sources and years has demonstrated that the contaminant/response is found n all formulations. Laboratory experiments indicate that MFO induction is rapid but deputation of the response is also very quick, i.e. a few days. Field studies have confirmed that the MFO induction is limited both temporally and spatial during a regular lampicide application in both caged and wild fish. Detailed TIE and GC-MS analysis of HPLC fractions has identified numerous contaminants in the bioactive fractions including a trifuoromethyl-chloro-nitro-dibenzodioxin. Synthesis of this compound has confirmed it's ability to cause MFO induction. Although the specific congener has not yet been identified the TIE approach was successful at characterizing the responsible chemical and providing remedial options.

TOXICITY IDENTIFICATION AT AN ALUMINUM SMELTER

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Due to intermittent toxicity of effluent discharged from ALCAN's Kitimat smelter, a Toxicity Identification Evaluation (TIE) project was initiated by the Company. The project's objectives were twofold, [1] determine the cause of toxicity, and [2] take action to remove the toxicity. The overall approach entailed using *Ceriodaphnia dubia* as surrogate test organisms and confirming the results with rainbow trout. Toxicity was removed by the chelating agent EDTA and was strongly dependent on hardness and, to a lesser extent, pH. Collectively, the data suggest that toxicity was due to a divalent cationic metal and that seasonal variations in discharge pH and hardness, as well as other water chemistry parameters, had appreciable effects on toxicity. This paper will present the study design, the use of surrogate test organisms, the TIE methods used, and the results. An innovative treatment strategy to eliminate toxicity will also be described.

TOXICITY IDENTIFICATION AND EVALUATION FOR THE PULP AND PAPER INDUSTRY. I. INSIGHTS FOR TOXICITY-FREE PULP AND PAPER MILL EFFLUENTS

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ABSTRACT

In 1994-95, a survey seeking information from Paprican member companies on the status of acute lethal toxicity of pulp and paper mill effluents was undertaken. Of the 74 mills that responded to a questionnaire, 35% (18 of 52) of the mills with secondary-treated effluent experienced from one to greater than four episodes of acute lethal toxicity in a two year period. Of these mills, half could attribute the toxicity to known factors. Based on the results of the survey and information in previous publications, the occurrence of effluent toxicity can be best controlled by tight operating conditions, minimization of spills and good housekeeping practises. If toxicity breakthroughs still occur, a series of steps can be taken by mill staff to identify the causative factors. These steps include ensuring that mortality of the test species was not caused by test conditions, an examination of the mill's record of operating conditions, and limited chemical analysis. A standardized toxicity identification evaluation (TIE) procedure, developed by the U.S. Environmental Protection Agency for industrial and municipal effluents, is also available. However, the TIE is usually done by consultants and its applicability for pulp and paper mill effluents has not yet been fully assessed.

RÉSUMÉ

En 1994-95, nous avons entrepris une enquête en vue de recueillir des renseignements auprès des sociétés membres de Paprican sur le statut de la toxicité létale aiguë dans les effluents des usines de pâtes et papiers. Des soixante-quatorze usines qui ont répondu à notre questionnaire, 35 pour cent (18 de 52) des usines dotées d'une installation de traitement secondaire des effluents ont indiqué avoir connu entre une et au-delà de quatre situtations de toxicité létale aiguë au cours d'une période de deux ans. Près de la moitié de ces usines ont indiqué qu'il leur était possible d'attribuer cette toxicité aiguë à des facteurs connus. En nous fondant sur les résultats de cette enquête et sur les renseignements puisés dans les publications antérieures, nous pouvons dire que l'occurence de la toxicité dans les effluents peut être le plus efficacement contrôlée en maintenant des conditions d'exploitation rigoureuses, en minimisant les répandages et en pronant de bonnes pratiques d'entretien. Si des problèmes de toxicité se présentent de nouveau, une série de mesures peut être prise par le personnel d'usine pour identifier les facteurs responsables de cet état de faits. Parmi ces mesures, notons l'étape

de vérification visant à établir que la mort de l'espèce analysée n'a pas été causée par les conditions de toxicité de l'essai, l'examen des archives de l'usine sur les conditions d'exploitation, et l'analyse chimmique limitée. Une méthode normalisée d'identification et d'évaluation de la toxicité (TIE) développée par l'EPA des États-Unis relativement aux effluents industriels et municipaux peut aussi être utilisée. Toutefois, la méthode TIE est habituellement réservée aux consultants et son application dans le cas des effluents des usines de pâtes et papiers n'a pas encore été entièrement évaluée.

INTRODUCTION

In 1992, the federal government revised the toxicity regulation for pulp and paper mill effluents (CFA, 1992). The revised regulation requires that effluents discharged by mills cause no acute lethal toxicity to rainbow trout (*Oncorhynchus mykiss*) in laboratory tests conducted according to standard protocols (EC, 1990a). For mills that were unable to comply with the regulation by December 31, 1993, there was a provision whereby the provincial and federal Ministers of the Environment could jointly extend a transitional authorization period for non-compliance up to December 31, 1995.

Testing with rainbow trout must be done once a month with undiluted (i.e., single concentration or 100% concentration) effluent that is discharged to the recipient. An effluent is considered acutely lethal, and thus fails the regulatory requirement, if at a concentration of 100% more than half of the rainbow trout die during a 96 h exposure period. In such cases, the testing frequency must be increased to once a week and the LC_{50} (i.e., effluent concentration causing 50% mortality during the 96 h exposure period) of the effluent must be determined. This requires that tests be conducted at five different effluent concentrations as opposed to the single concentration regulatory test.

The revised regulations also require mills to conduct acute lethal tests with the water flea *Daphnia magna*. These tests must be done once a week according to standard protocols (EC, 1990b) and are intended for monitoring purposes only. However, if an effluent is found to be acutely lethal to *D. magna*, that is if the effluent causes more than 50% of the organisms to die during a 48 h exposure period, additional toxicity monitoring must begin. This includes a 96 h LC_{50} rainbow trout test with an effluent sample collected without delay as well as 48 h LC_{50} tests with *D. magna* conducted every three days. In the event of failure in either the trout or the *D. magna* test, the increased schedule of testing must be continued until three consecutive effluents meet the regulatory limit. During episodes of non compliance, the mills also have to provide the authorities with a plan of action for remedying the situation.

In Canada, most mills achieve compliance with the regulatory limit by secondary treatment of their effluents (Johnson, 1976; Leach et al. 1977; Leach et al., 1978; McCubbin, 1983; Bailey, 1994). Almost all secondary treatment plants in Canada are aerobic (mostly aerated lagoons and activated sludge plants) (Bailey, 1994) with a few mills using anaerobic/aerobic options (Wilson et al., 1987; MacLean et al. 1990; Bailey, 1994). Can the industry meet the challenge of the toxicity regulation on a consistent basis solely by secondary treatment of effluent? According to a 1977 report, aerated lagoons in British Columbia failed to eliminate acute lethal toxicity 20% of the time (Leach et al., 1977). So far, the situation in the 1990s is not well documented. If occasional toxicity breakthroughs occur despite effluent treatment, as was reported in the 1970s, are there steps that can be taken to identify the causes, quickly remedy the situation and return to non-lethal status? While answers to these questions will no doubt emerge in the future, there is a need to know, as quickly as possible, if effluent acute lethal toxicity is problematic for the Canadian pulp and paper industry and if research and technical support will be required.

The intent of this report is to describe the results of a survey seeking information from Paprican member companies on the status of acute lethal toxicity of pulp and paper mill effluents in late 1995. In particular, attention was focussed on the ability of secondary treatment installations to eliminate effluent toxicity on a consistent basis. The survey was not meant to be an indicator of regulatory compliance,

APPENDIX I
Questionnaire on the Acute Lethal Toxicity of Pulp and Paper Mill Effluents

Acute Lethal Toxicity Survey

Date:_____

Mill Name:_____

Location:_____

Name of Person Completing Survey:_____

Telephone No.:_____

- In the last two years, has your mill experienced episodes of acute lethal toxicity (i.e., LC50 < 100%) of its final mill effluent with respect to:

  Trout ..... Yes ☐ No ☐

  Daphnia ..... Yes ☐ No ☐

- If yes:

  State frequency of toxicity occurrences ..... Once/year ☐

  2-3 times/year ☐

  ≥ 4 times/year ☐

  Did toxicity occur more often during ..... Spring ☐

  Summer ☐

  Fall ☐

  Winter ☐

  Random ☐

  Was the cause of toxicity identified? ..... Yes ☐

  No ☐

- Description of your mill's treatment system ..... Primary only ☐

  Aerated lagoon ☐

  Activated sludge ☐

  Other ☐

since during the time of the survey many mills were still in the transitional period of achieving compliance. Information on the potential causes of effluent toxicity and steps that may be taken to determine these causes were also compiled. This was done to suggest a course of action for mill staff in the event of a toxicity episode.

SURVEY OUTLINE: During September 1994, a short questionnaire (see Appendix I) was sent to Paprican's maintaining member companies requesting information about incidents of effluent acute lethal toxicity during the past two years. If toxicity episodes had occurred, the questionnaire asked about their frequency and whether the causes for toxicity were identified. Information about the type of effluent treatment employed by the mills was also sought. By December 1994, 72 out of the 88 mills surveyed (82%) had responded to the questionnaire. More than half of the responding mills (i.e., 38) were anticipating the start-up of secondary treatment facilities in the upcoming year (i.e., in 1995). Therefore, in late 1995 the questionnaire was resent to those mills, as well as the mills which had not responded to the 1994 questionnaire, to obtain a more complete and up-to-date description of the situation just prior to the deadline for meeting the regulatory requirement. The questionnaire did not request information on effluent sublethal toxicity. Therefore, in this report, any further reference to toxicity indicates acute lethal toxicity.

SURVEY RESULTS: In total, on the basis of both surveys, information was obtained from 74 of the 88 mills which were sent questionnaires. For mills that responded in 1994 and late 1995, 52 had secondary treatment facilities, 20 employed primary treatment only and two facilities anticipated the use of other options such as evaporation or internal water reduction strategies leading to elimination of liquid discharges. A breakdown of the information extracted from the returned questionnaires is given in Fig. 1.

Primary-Treated Effluents: The effluents from the 22 mills with only primary treatment were acutely lethal (Fig. 1) to both rainbow trout and *D. magna* (i.e., LC_{50} <100%). Of these mills, nine indicated that secondary treatment plants would be operational in 1996, while two mills have chosen other control options. The remaining 11 mills did not indicate their plans concerning strategies for dealing with this issue.

Secondary-Treated Effluents: During a two year period, 18 out of 52 (35%) mills with secondary treatment of effluent experienced from one to more than four acute lethal toxicity episodes (Fig. 1). For 13 of these mills, the frequency of these episodes was low (one or two times), while for the remaining five mills it occurred more frequently (i.e., >4 times). The effluent was toxic to both rainbow trout and *D. magna* in 13 cases, toxic only to trout in four cases and toxic to only *D. magna* in one instance.

Of the mills with aerated lagoon and activated sludge treatment systems, 52% and 21%, respectively, reported cases of acute lethal toxicity (Fig. 2a). The better performance of the activated sludge treatment plants possibly reflects the greater control which is available for this type of system. Hence, any mill upsets or spills are more easily handled and controlled. However, general conclusions about the efficiency of different secondary treatment plants should not be made on the basis of only this survey. The four mills with sequential batch reactors (SBRs) did not report any toxicity. These were new installations that had been in operation for only three to five months and only a few toxicity tests had been performed. Toxicity was reported for the only mill in the survey operating an anaerobic/aerobic treatment system.

For mills having secondary treatment systems, a breakdown of the survey results with respect to the different manufacturing processes is given in Fig. 2b. There was no indication that any type of manufacturing process resulted in discharges substantially more prone to cause toxicity. The role of water usage, which may be an important factor in final mill effluent toxicity, was not appraised in this survey. However, the toxicity of mill effluents depends on the concentration of individual compounds present. Any reduction in water usage by mills, a current trend in the Canadian industry working

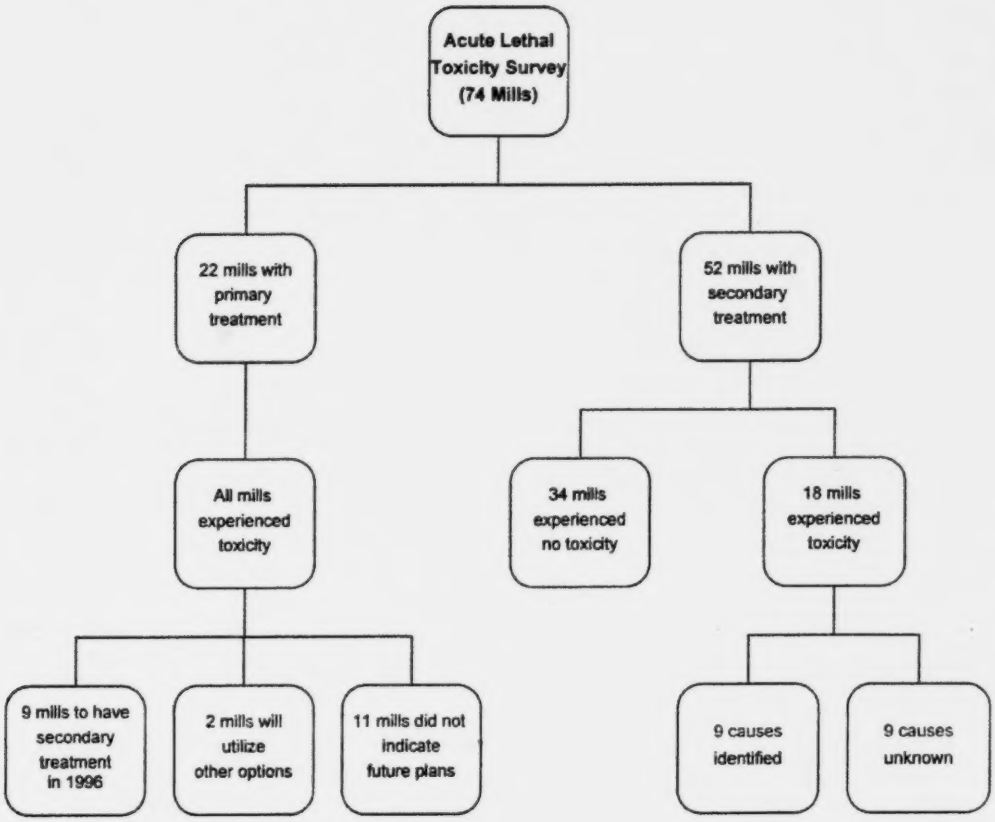
Acute Lethal Toxicity Survey (74 Mills)
22 mills with primary treatment
52 mills with secondary treatment
All mills experienced toxicity
34 mills experienced no toxicity
18 mills experienced toxicity
9 mills to have secondary treatment in 1996
2 mills will utilize other options
11 mills did not indicate future plans
9 causes identified
9 causes unknown

Fig. 1. Results of acute toxicity survey of Canadian pulp and paper mills.

towards progressive systems closure, may result in a higherrial causing toxicity in the final effluent. While the precise effects of water reduction at a particular mill is difficult to predict, the increased likelihood of effluent toxicity exists. Besides water usage, other factors that influence the final mill effluent toxicity include in-plant and effluent treatment practises. As pointed out by Bonsor et al. (1988), mills that conserve water tend to have good control practices, resulting in reduced chemical usage and thus the compounds causing toxicity are curbed at source. In the case of secondary treatment, reduced water usage likely increases the retention time. Therefore, while the concentration of effluent components is increased, the longer retention time provides better treatment efficiency. Alternatively, concentrations of effluent components may become so great they become toxic to the microorganisms present in the secondary treatment systems. In view of this, the point at which these factors become important will no doubt be site-specific and require assessment on a case-by-case basis. In a pilot study with bleaching effluent at a Canadian kraft mill, a 25 to 30% reduction of water did not increase the risk of effluent toxicity after treatment, but when water flow was reduced by 50% the regulatory toxicity limit could not be met (Wohlgemuth et al., 1996). One way to address the water usage issue would be to change the toxicity regulation from a simple pass and fail requirement to one based on water consumption and production (Bonsor et al., 1988). This is already done for the other effluent parameters which are regulated. For example, the regulatory limits for biochemical oxygen demand (BOD) and suspended solids are set as kg of material discharged per tonne of manufactured product.

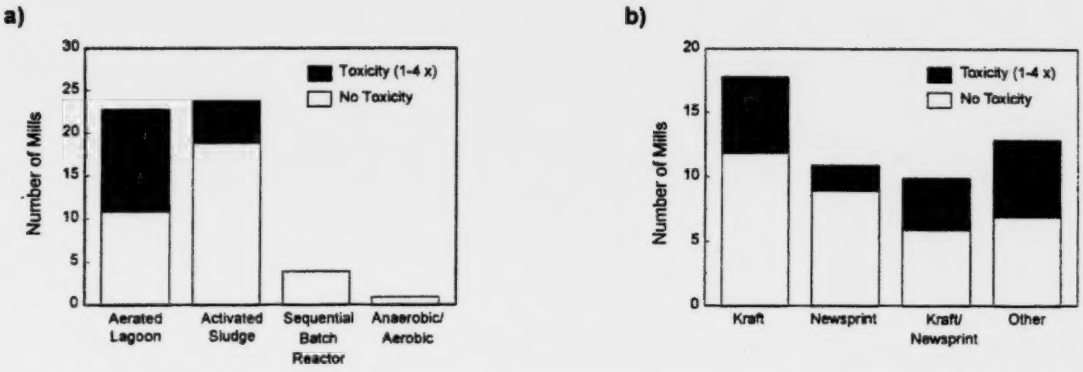

Fig. 2. Compilation of acute toxicity survey results for secondary-treated mill effluents according to: (a) treatment system, and (b) process type.

Identified Causes of Acute Lethal Toxicity

The 18 mills where secondary-treated effluents exhibited occasional toxicity were contacted individually to determine if the causes for the toxicity were known. In half of the cases, toxicity incidences could be attributed to known factors affecting the treatment system or mill operating conditions. These are listed below.

Failures related to treatment system included [1] in three instances, effluent toxicity occurred because the aerated lagoons were not operating efficiently due to very cold weather conditions. [2] In one case, the short retention time (as short as 8 h) of the effluent in the aerated lagoon, caused by a build-up of sludge in the treatment system, resulted in toxicity breakthrough. [3] At one mill, the effluent in the primary clarifier became anaerobic. This introduced a shock into the aerobic system and reduced treatment efficiency.

Failures related to operating conditions included [1] in two cases, the cause of the toxicity was identified as a foam spill or the lack of foam skimming equipment. The resin acids in the foam were the suspected cause of the toxicity. [2] For two mills, the use of freshly cut wood appeared to be correlated to the toxic events. The levels of extractives in fresh wood are higher than in wood that has been stored for several months (Assarsson and Croon 1963; Levitin, 1967; Rogers et al., 1971; Wong et al., 1980; Wong, 1981; O'Conner et al., 1992) and it seems that the treatment systems were incapable of handling the increased load. [3] At one mill, toxicity occurred during start-up or shutdown when operating conditions fluctuated widely and the treatment system received no feed for a period of time.

What to do When Effluent Acute Lethal Toxicity Occur: For some of the mills with secondary effluent treatment participating in the survey, it appears that the toxicity episodes occurred under extreme conditions (e.g., intense cold, start-up difficulties) or during periods of atypical operation. For these mills, the normal operating mode did not result in acutely lethal effluent. Clearly, as also indicated by previous authors (Reibel and Lavalée, 1995; Reibel et al., 1996), attempts to prevent the possibility of toxicity incidents through consistent and tightly controlled operating conditions is the best course of action. For several other mills taking part in the survey, however, the cause(s) of the acute lethal toxicity was unknown. It is possible that the reasons for toxicity were similar to those presented above. Alternatively, other factors may have been responsible. In any case, when incidents of effluent acute lethal toxicity are observed, steps need to be taken to remedy the situation. These may include common-sense steps undertaken by mill staff or a formal toxicity reduction/toxicity identification evaluation (TR/TIE) scheme developed by the U.S. Environmental Protection Agency (EPA) (Mount,

1988; Mount and Anderson-Carnahan, 1988a; 1988b; Fava et al. 1989) and usually completed by consultants. A complete TR/TIE study, which involves chemical analyses as well as toxicity testing, can be very time consuming, expensive (e.g., as much as hundreds of thousands of dollars) and carries no guarantee of success (Reibel and Lavalée, 1995; Reibel et al.,1996). Therefore, prior to commissioning a full-scale TR/TIE study, preliminary in-house investigations by mill staff may be useful.

Insights and Recommendations for Mill Staff: Our insights and recommendations for a course of action to be considered by mill personnel during effluent toxicity occurrences are outlined in Fig. 3 and elaborated upon in the following sections. The underlying assumption is that staff know the operating conditions at their mill intimately and are thus most likely to identify the source of the problem as long as they are also knowledgeable about toxicology and toxicity testing. To be successful, individuals from different departments (e.g., wood handling, bleaching) will have to share their expertise and cooperate.

The information in Fig. 3 includes certain elements of the TR/TIE methodology as well as other common-sense steps based, in part, on our experience at Paprican. First, it should be established that the observed fish mortality is indeed caused by effluent toxicity and not laboratory testing conditions. If the mortality is confirmed to be related to the effluent, information from the toxicity test reports regarding symptoms exhibited by the fish prior to death, may be related to specific effluent components. A thorough evaluation of mill operating conditions and effluent characteristics (e.g., BOD), including the use of additives, is also a crucial undertaking.

It is important to bear in mind that each mill is unique (Axegård et al., 1993) and thus the procedure indicated in Fig. 3 is not necessarily meant to be all inclusive and may have to be modified to account for site-specific differences. Similar insights and recommendations have been presented by other authors (Reibel and Lavalée, 1995; Reibel et al., 1996) and these publications should also be consulted when initiating such work. We are also providing additional information in 'Further insights and recommendation' section of this report for those who need to direct more elaborate studies. Finally, the course of action available to mill staff will surely increase and improve with time as experiences are shared and the results of research activities in this field are presented.

Evaluation of Laboratory Testing Conditions (Fig. 3a)

Effluent samples should only be tested in reputable laboratories. The Canadian Association of Environmental Analytical Laboratories (CAEAL) has implemented a laboratory accreditation program and the use of accredited laboratories is highly recommended. Laboratories must adhere to strict guidelines in order to be accredited. In addition, records of toxicity test results with reference compounds, compounds for which toxic levels are well known, must be available and must indicate little deviation above what is expected to occur as a consequence of natural variation. The Directory of Accredited Laboratories is published every year and the updated directory may be obtained from the CAEAL offices in Ottawa (phone: 613-233-5300). In Quebec, the Ministère de l'Environnement et de la Faune, Direction des Laboratoires (418-528-0411), does accrediting and publishes a list of these laboratories on a regular basis.

On most occasions, toxicity tests with effluents are conducted by contract laboratories in strict accordance with Environment Canada protocols (EC, 1990a, 1990b). However, in some instances and for a variety of reasons (e.g., unavailability of fish of a certain size), tests are not done according to regulatory methodology (e.g., unacceptable loading density, insufficient acclimation of fish to laboratory conditions). In these cases, fish sensitivity and, hence, survival can be affected. Therefore, the toxicity test reports should be carefully reviewed to determine if the test was done in an appropriate manner. In addition to the above mentioned items, special attention should be given to the pH and dissolved oxygen levels of the effluent during the test. If the dissolved oxygen falls below 5 mg/L and the pH falls outside the range of 6-9, these conditions could contribute to the fish mortality.

Even if laboratories are accredited and conduct tests according to accepted protocols, there can be substantial variations in mortality rates of organisms exposed to effluents in different laboratories and even in tests conducted with the same effluent in one laboratory (Parkhurst et al., 1992; Warren-Hicks and Parkhurst, 1992; Orr and Craig, 1996). For example, in the case of a secondary-treated bleached kraft mill effluent (BKME), three laboratories found no acute lethal toxicity to *D. magna* (i.e., 48 h LC_{50} >100%), but one laboratory reported a 48 h LC_{50} of approximately 80% (Kovas and Ferguson, 1990). Similarly, significant variations have been reported for the results of rainbow trout tests with selected wood extractives (Chung et al., 1975). Consequently, when effluent toxicity occurs, particularly marginal toxicity, the possibility that this was the consequence of the imprecision associated with the test should be considered (Orr, 1993; Orr and Craig, 1996). Sending sub-samples of the same effluent to different laboratories for testing is helpful in establishing that the observed toxicity is real and not laboratory dependent. The process could be facilitated if a sub-sample of the effluent originally sent out for toxicity testing was kept at <5°C. The need for an archived sample is often necessary because effluent sampled subsequently is no longer toxic.

Interlaboratory differences occur due to a variety of abiotic and biotic factors. One important factor in tests with undiluted effluents is the characteristics of the water used for holding and culturing the test organisms in the laboratory. While Environment Canada protocols for acute lethal testing are quite detailed (EC, 1990a, 1990b), for reasons of practicality, they do allow for some variations regarding water quality. As an example, the water for holding and acclimating rainbow trout can have a pH in the range of 6.0-8.5. However, the optimum pH for salmonids is 7.5 to 8.5 and water with a pH in the range of 6.0 to 6.5 appears to reduce the protective mucus slime on the fish (Klontz et al., 1979). The hardness and alkalinity of the water can also affect the well-being of fish, but there are no specific requirements for these parameters in the regulatory test protocols. Low alkalinity (<20 mg/L as $CaCO_3$), which is associated with waters of pH below 7, results in slow growth (Klontz et al., 1979). The calcium content of the water usually increases with hardness. Fish need calcium from the water, in addition to calcium obtained through the diet, for respiratory and osmoregulatory activities (Klontz et al., 1979). Therefore, while rainbow trout kept in waters with low pH (< 7.5), alkalinity and hardness are held under conditions meeting the regulatory test criteria, the sensitivity of such fish to effluents may be greater than if they were kept under more optimal conditions.

The characteristics of the water used for holding the organisms in the laboratory can influence the test results in still another manner. If the pH or the hardness of the laboratory water differs substantially from the pH or hardness of the effluent tested, placing the organisms in the effluent may result in stress, such as osmoregulatory stress. Therefore, it would be in the mill's interest to choose a laboratory that has water characteristics similar to that of the effluent being tested.

Symptoms of Toxicity Associated with Specific Agents (Fig. 3b)

Even though the endpoint of the regulatory test with rainbow trout is mortality, the behaviour and appearance of the fish prior to death varies, depending on the material causing toxicity. Therefore, careful observation and recording the symptoms exhibited by the fish can provide clues for potential causative agents. Certainly, such observations should accompany toxicity test reports and should be scrutinized by mill personnel. Examples of typical symptoms associated with a limited number of compounds, previously identified in mill effluents as causing toxicity, are given below (a more detailed discussion of some compounds is provided in section on 'Further insights and recommendations').

Ammonia: Based on visual observations in our laboratory, there is a progression of symptoms associated with ammonia toxicity. The fish become darkened, sluggish, sink to the bottom of the container and lose equilibrium. They do not appear to be under great distress and once the initial symptoms appear, mortality can occur rapidly. Another indication that ammonia may cause mortality is related to the time when fish die. In our experience, pulp and paper mill effluents cause fish mortality usually between the first 12 to 24 h of testing. When mortality is observed on the third or fourth day,

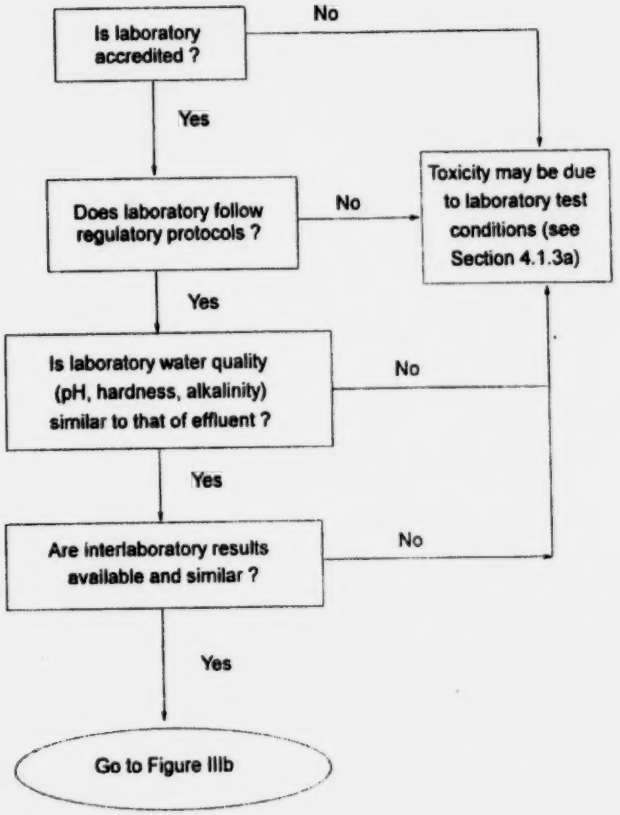

Figure III. *Summary of insights and recommendations for dealing with acute lethal toxicity of pulp and paper mill effluents.*
a. Evaluation of testing laboratory (Section 4.1.1).

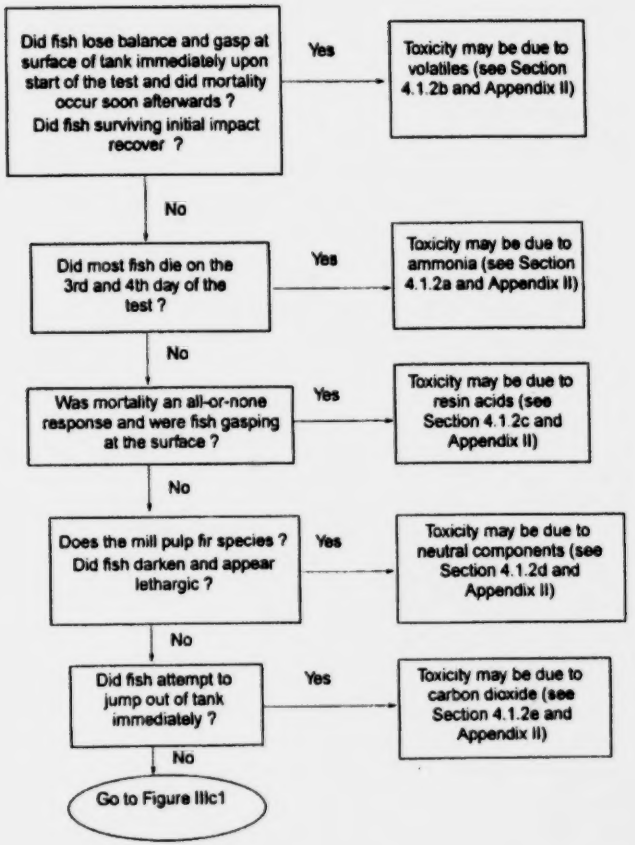
Did fish lose balance and gasp at surface of tank immediately upon start of the test and did mortality occur soon afterwards ?
Did fish surviving initial impact recover ?
Yes
Toxicity may be due to volatiles (see Section 4.1.2b and Appendix II)
No
Did most fish die on the 3rd and 4th day of the test ?
Yes
Toxicity may be due to ammonia (see Section 4.1.2a and Appendix II)
No
Was mortality an all-or-none response and were fish gasping at the surface ?
Yes
Toxicity may be due to resin acids (see Section 4.1.2c and Appendix II)
No
Does the mill pulp fir species ? Did fish darken and appear lethargic ?
Yes
Toxicity may be due to neutral components (see Section 4.1.2d and Appendix II)
No
Did fish attempt to jump out of tank immediately ?
Yes
Toxicity may be due to carbon dioxide (see Section 4.1.2e and Appendix II)
No
Go to Figure IIIc1

Figure III. *Summary of insights and recommendations for dealing with acute lethal toxicity of pulp and paper mill effluents.*
b. Symptoms of toxicity (Section 4.1.2).

it often occurs as a result of the formation of ammonia during the test or as a result of rising pH increasing the toxicity of the ammonia present. During testing, effluents must be aerated to ensure that fish will have sufficient dissolved oxygen to survive. The aeration of the effluent, stripping out carbon dioxide, results in a rise of pH (Schmell et al., 1990). Since ammonia becomes more toxic as pH increases(Thurston et al., 1981), ammonia levels which were not toxic at the start become toxic as the test progresses.

Volatiles: In addition to obvious odour, the most distinct feature of toxicity caused by volatile material such as hydrogen sulphide is rapid mortality (<1 h). The fish lose their balance immediately upon placement in the effluent. The individuals that do not die within the first few hours eventually recover, probably because the volatiles are stripped out of the solution as a consequence of aeration. Fish will also appear to be gasping for air at the surface of the effluent, although this is also a symptom for many other types of toxicants affecting the respiratory system. Since xenobiotics enter the fish primarily through the gills, the gill structure is often damaged resulting in reduced ability to extract dissolved oxygen from the solution.

Resin acids: Fish death caused by resin acids occurs without a long period of severe distress preceding mortality (Rogers and Mahood, 1974). Nevertheless, fish are seen at the surface of the effluent tested since resin acids are suspected to damage the gills (BCR, 1978; Tuurala and Soivio, 1982). Fish exposed to lethal levels of dehydroabietic acid have shown weakened schooling behaviour and have appeared lethargic as evidenced by virtually no reaction to disturbances (Rogers et al., 1975; Kruzynski, 1979). The fish eventually lose muscular coordination leading to spasms and loss of equilibrium just prior to death.

Fish exposed to lethal solutions of dehydroabietic acid in our laboratory exhibit the following symptoms in sequence: slight loss of equilibrium, gulping at the surface, movement of all the fish to the solution-air interface, increased loss of equilibrium, enlargement of the swim bladder resulting in distended abdomen, sink to the bottom, curvature of the body, darkened colouration and reduced respiration. Another distinct feature of resin acid toxicity is an 'all-or-none' type of mortality. This means that partial mortality will be rarely observed as either all of the fish or none of the fish die during the exposure period.

Neutrals: Neutral compounds such as diterpene alcohols and aldehydes were observed to cause severe stress in fish (e.g., loss of balance) prior to mortality (Rogers and Mahood, 1974). Juvabione and juvabione-type compounds, which can be present in mechanical pulping and sulphite mill effluents where balsam fir is the wood furnish, cause fish to become lethargic and their scales to darken (Leach et al., 1975). Also, as for dehydroabietic acid, the swim bladders and thus the abdomens of the fish exposed to these types of compounds are often distended.

Carbon dioxide: Symptoms of carbon dioxide poisoning in fish include erratic swimming in quick bursts, including attempts to jump out of the tank, and gulping at the effluent surface (Kuenzi and Thiel, 1980). These symptoms occur instantaneously. Based on our observations, this is followed by the fish sinking to the bottom of the container and taking on a rigid crescent shaped appearance. In many cases, even though the fish appear dead (no obvious gill movement) they readily recover if the carbon dioxide is driven off from the solution.

Evaluation of Mill Operating Conditions and Effluent Treatment (Fig. 3c)

Once it is established that the observed mortality in organisms exposed to effluent is not the consequence of the testing circumstances, an examination of the record of historical effluent quality, operating conditions and simple inspection of certain areas of the mill can be helpful. Comparing the quality of the untreated effluent and the treated effluents, based on conventional parameters such as BOD and COD, before and after the toxicity occurrence can provide direction whether the problem

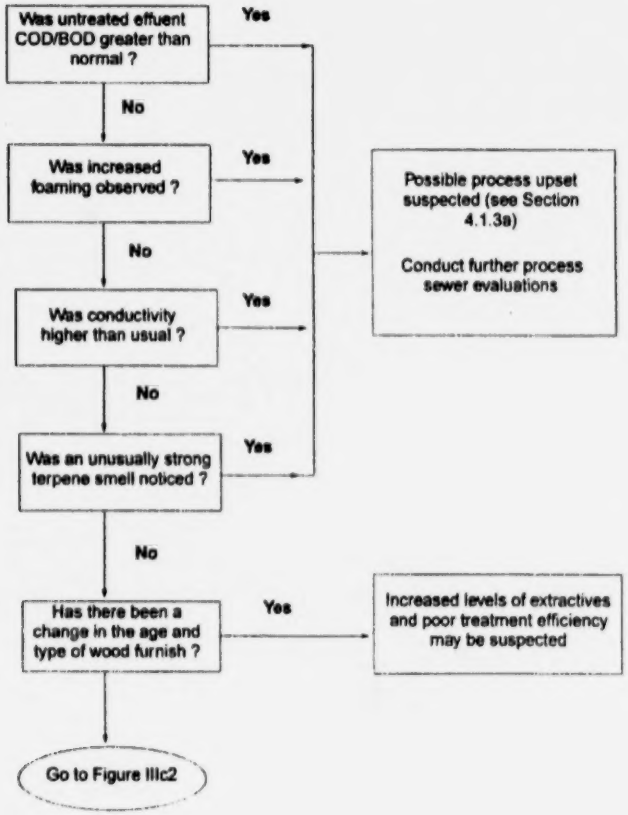
Was untreated effluent COD/BOD greater than normal ?
Yes
No
Was increased foaming observed ?
Yes
No
Was conductivity higher than usual ?
Yes
No
Was an unusually strong terpene smell noticed ?
Yes
No
Has there been a change in the age and type of wood furnish ?
Yes
Go to Figure IIIc2
Possible process upset suspected (see Section 4.1.3a)
Conduct further process sewer evaluations
Increased levels of extractives and poor treatment efficiency may be suspected

Figure III. *Summary of insights and recommendations for dealing with acute lethal toxicity of pulp and paper mill effluents.*
c1. Evaluation of mill operating conditions (Section 4.1.2), combined mill effluent prior to treatment.

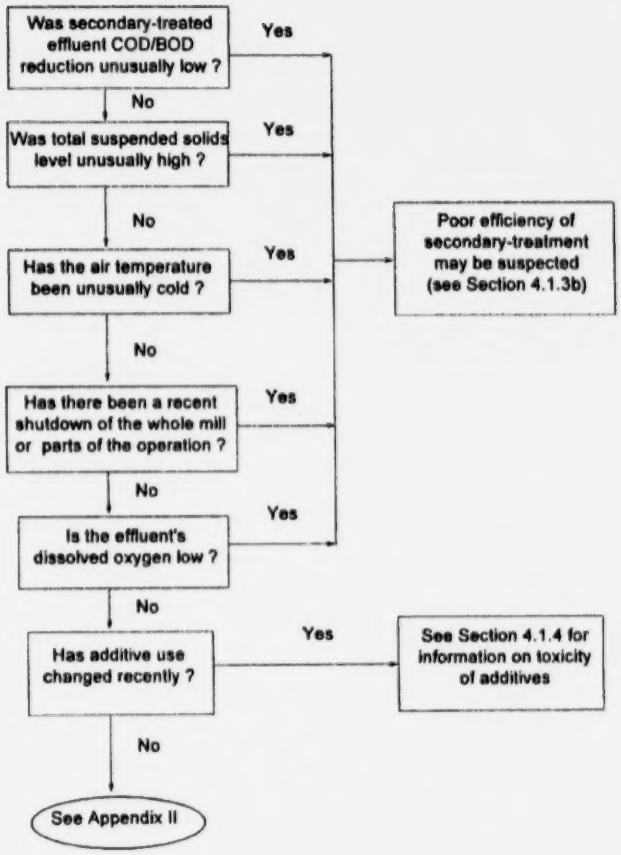
Was secondary-treated effluent COD/BOD reduction unusually low ?
Yes
No
Was total suspended solids level unusually high ?
Yes
No
Has the air temperature been unusually cold ?
Yes
No
Has there been a recent shutdown of the whole mill or parts of the operation ?
Yes
No
Is the effluent's dissolved oxygen low ?
Yes
No
Has additive use changed recently ?
Yes
No
See Appendix II
Poor efficiency of secondary-treatment may be suspected (see Section 4.1.3b)
See Section 4.1.4 for information on toxicity of additives

Figure III. *Summary of insights and recommendations for dealing with acute lethal toxicity of pulp and paper mill effluents.*
c2. Evaluation of mill operating conditions (Section 4.1.2), secondary-treated effluent.

originates within the mill or in the treatment system. This is not based on any relationship between these conventional parameters (e.g., BOD) and toxicity (Howard and Walden, 1971), but is simply an indication that the effluent quality has changed. For example, if effluent characteristics (based on conventional parameters) prior to treatment are essentially similar before and after a toxic episode, then a malfunction in the treatment system may be suspected. If, on the other hand, the combined mill effluent prior to treatment is substantially different from the norm, then obviously conditions within the mill have changed and are likely responsible for the toxicity breakthrough. In these cases investigations should then focus on the characteristics of various process sewers to pinpoint the actual trouble spot.

Process Upsets (Fig. 3c1): In some instances, as indicated from our survey and a previous report (Leach et al., 1977), the toxicity could be due to process upsets such as condensate spills or black liquor losses which overload the treatment system. These may be easily detected from records of monitoring the characteristics of process sewers. For example, potential indications of black liquor losses should be considered when increased conductivity are detected in the effluent. A condensate spill might lead to a low BOD and COD removal in the treatment plant and a turpentine effluent odour due to the presence of terpenes.

At times, and depending on the wood furnish used by the mill, increased foaming may be observed in the treatment system. A change in the age of the wood furnish can influence the extractives levels in the effluent, its overall characteristics and treatability (Assarsson and Croon, 1963; Rogers et al., 1971; Wong, 1981; Dorica et al., 1985; O'Conner et al. 1992) and thus should be a suspected factor responsible for toxicity. Good inventory and record keeping of the wood furnish may be helpful in such cases.

Problems with Effluent Treatment (Fig. 3c2): Effluent toxicity can result from inefficient treatment, which may in part be due to extremely cold weather conditions, increased organic load, foam collapse, non-optimal nutrient addition, changes in sludge age, insufficient mixing and short retention time or effluent channelling (aerated lagoons) (Leach et al., 1977; Walden and Howard, 1977; Leach and Thakore, 1978; Walden and Howard, 1981). Indications of treatment problems could be low BOD and COD removal, increased suspended solids, ammonia build-up (due to biomass decomposition) and unusually high levels of wood extractives, such as resin and fatty acids (RFAs) in the treated effluents (see Appendix II).

Inventory of Additives Use (Fig. 3c2): While most additives (e.g., biocides) in the final effluent are not present in sufficient quantities to cause acute lethal toxicity (Poole et al. 1978; Milanova and Sitholé, 1995b; Milanova et al., 1995), mill operators should review the use of the amounts and types of additives just before and during the episode of toxicity failure. Specific biocides and polymers have been identified as causes of toxicity in the effluent discharged by two fine paper mills (Fein et al., 1994). Information on the additives commonly used by pulp and paper mills in Canada have recently been compiled in a Paprican report (Sitholé and Milanova, 1994). In addition, Paprican researchers have conducted toxicity tests with several dyes and biocides lacking adequate toxicological data (Milanova et al., 1995, Milanova and Sitholé, 1995b). Methods for analysing effluents for certain additives were also developed (Milanova and Sitholé, 1995a).

Further Insights and Recommendations: If the steps outlined for mill staff in the above sections have not identified the cause of the toxicity, additional steps need to be taken. These will likely involve consultants and/or contract laboratories. The insights and recommendations in the following sections are meant to be helpful for such investigations.

Chemical Analysis for Selected Effluent Components

Indiscriminate chemical analysis of effluent is not recommended. Nevertheless, analyzing effluent samples for selected components previously identified to be responsible for toxicity may be useful in

certain cases. The decision to analyze for selected components should be based on a careful review of the operating conditions, including a knowledge of the wood furnish used.

Wood Extractives: To date, most of the acute lethal toxicity of pulp and paper mill effluents prior to secondary treatment has been attributed to wood extractives (Rogers, 1971; Rogers and Mahood, 1974; Leach and Thakore, 1975; Walden, 1976; Leach et al., 1977; Chandrasekaran et al., 1978; Leach and Thakore, 1978; Rogers et al., 1979; Walden and Howard, 1981; Priha and Talka, 1986; McLeay, 1987; Young et al., 1990). Because the toxicity thresholds for many of the wood extractives are known (Leach et al., 1975; Walden, 1976; Leach and Thakore, 1978; McLeay, 1987), chemical analysis of effluents for these compounds is a logical starting point for investigations.

Secondary treatment of effluents readily degrades most of the wood-related compounds responsible for acute lethal toxicity (Rogers and Mahood, 1974; Rogers et al., 1975; Leach et al., 1977; Chandrasekaran et al., 1978; Easty et al.. 1978; Leach and Thakore, 1978; Walden and Howard, 1981; Priha and Talka, 1986; Gibbons et al., 1992). In fact, since the resin acids have often been cited as the major source of effluent toxicity (Howard and Walden, 1971; Rogers et al., 1975; Leach and Thakore, 1978; Priha and Talka, 1986), the success of detoxification has frequently been linked specifically to the removal of resin acids by secondary treatment (Chen et al., 1974; Mueller and Walden, 1974; Chung et al., 1975; Rogers and Mahood, 1974; Leach and Thakore, 1975; Leach et al., 1977). This has led to the commonly believed generalization that many instances of effluent toxicity can be attributed to the breakthrough of resin acids. However, this is not necessarily always the case. For one thing, resin acids are not expected to be present in effluents discharged by all mills since not all of the mills use a wood furnish containing resin acids. For example, mills pulping hardwoods do not discharge resin acids in the effluent. Trace amounts of resin acids, mostly dehydroabietic acid (DHAA), have been detected in some effluents with a hardwood furnish (McKaque et al., 1977; O'Conner et al., 1992), but the quantities were so minute that they could not account for any of the observed toxicity. Lignans, consisting of phenylpropane dimers (O'Conner et al., 1992), were found to account for the toxicity of effluents from mechanical pulping of hemlock (Solinas and Tustall, 1976). Clearly, the nature and, hence, the toxicity of the extractives is dependent on the type of wood species utilized by the mill (Rogers and Mahood, 1974; Leach and Thakore, 1978; O'Conner et al., 1992). Resin acids are expected in effluents mostly from the pulping of various pine, spruce and, to a lesser extent, fir species (Voss, 1987; O'Conner et al., 1992). When the furnish at the mill consists of these species, monitoring the final effluent for resin acids is a useful undertaking (Shepard et al., 1996). If the concentrations of resin acids or other wood extractives are present at levels causing mortality, a change in the age of the wood furnish or malfunctioning of the secondary treatment system should be suspected.

Other Effluent Components: There are reports of instances when effluents from mills pulping resinous softwoods contain no appreciable amounts of wood extractives after secondary treatment and still cause fish mortality (Rogers and Mahood, 1974; Cook and Chandrasekaran, 1986; Priha and Talka, 1986). For these cases, ammonia (Servizi and Gordon, 1986; Schnell et al., 1990), volatile organic material (when concentrations exceeded 300 µg/L) and carbon dioxide (Kuenzi and Thiel, 1980) have been found to be or suspected to be the causative agents. Ammonia levels in the effluent may reach toxic levels when nitrogen is added to ensure optimum functioning of the secondary treatment system. Several authors (Servizi and Gordon, 1986; Schnell et al., 1990) have also noted that lethal levels of ammonia (and less frequently nitrites from the oxidation of ammonia) were not present at the time of discharge but formed during effluent shipping or testing. At these times, the toxic concentrations of ammonia were likely formed as a consequence of endogenous metabolism of the biosolids (Servizi and Gordon, 1986). This phenomenon has been most often observed with secondary-treated newsprint (e.g., TMP, CTMP) effluents. Formation of volatile material in the effluent, such as hydrogen sulfide, can occur if anaerobic conditions develop during primary treatment or during shipment of secondary treated-effluent. High levels of carbon dioxide have been detected in an oxygen activated treatment plant as a consequence of very high rate biological activity when the treatment plant was covered and pressurized (Kuenzi and Thiel, 1980).

Complicating Factors

While analysis of effluents for selected components is often a useful undertaking, due to a variety of complicating factors, interpreting the results of such analysis needs to be done with caution. Previous efforts to predict effluent toxicity on the basis of chemical analyses were only partially successful. For example, Chung et al. (1975) attempted to develop a rapid chemical procedure to predict effluent toxicity. The procedure involved adsorption of effluent components on to polymeric resin, solvent elution and gas chromatographic analysis. The whole process was said to take approximately 1.5 h (substantially less time than the 4 d required for a trout test) and results for more than 60 effluent samples indicated that agreement between predicted toxicity based on chemical analysis and actual trout bioassays was within 30% for 76% of the samples. Because of this, the chemical analysis approach was suggested to be a useful supplement but not a substitute for direct toxicity testing. We agree with this assessment and offer our insights for factors that complicate prediction of effluent toxicity solely on the basis of chemical analysis.

As pointed out by Chung et al. (1975), there can be significant differences in the acute lethal toxicity thresholds for many of the effluent components reported in the literature. This variation is, in part, due to the natural variation associated with biological testing described earlier as well as differences in the sensitivities of fish, possibly related to genetics (Chung et al., 1975). Furthermore, the results of toxicity tests with individual compounds is not always applicable for mill effluents (Orr, 1993; Reibel and Lavalée, 1995; Reibel et al., 1996). The effluent matrix (e.g., suspended solids, pH) is doubtless different from the of the compound was tested. The different matrix can alter the toxicological properties of the compound in question as well as its solubility and bioavailability (Kovacs and Ferguson, 1990; Orr, 1993). For example, the toxicity of resin acids and ammonia can vary substantially with pH (McLeay et al., 1979; Thurston et al., 1981; Martel, 1990). The 96 h LC_{50} of DHAA (one of the most common resin acids in softwood mill effluents) to rainbow trout was reported to range from 0.77 to 1.2 mg/L (Taylor and Yeager, 1987). These results were obtained from assays conducted in British Columbia where the dilution water used for preparing test solutions and fish holding was relatively soft (i.e., <47 mg/L as $CaCO_3$) and had pH ranging from 6.4 to 7.0. When tests were conducted in water with a pH of 7.5 and 8.5 (i.e., dilution waters more common in central Canadian laboratories), the 96 h LC_{50} of DHAA to rainbow trout was reported to be 2.2 mg/L and 16 mg/L, respectively (Kovacs and Ferguson, 1990). Similar differences in toxicity of DHAA were observed for *D. magna* and fathead minnows tested in waters with different pHs (Zanella, 1983; Kovacs and Ferguson, 1990). The unionized forms of DHAA, which are favoured by low pH, are more readily absorbed by aquatic organisms and therefore are more toxic than the ionized forms. As discussed in section on 'Symptoms of toxicity associated with specific agents,' in contrast to resin acids, ammonia becomes more toxic as pH increases. A final complicating factor is the possibility for toxicity additivity in effluents which contain numerous compounds (Reibel and Lavalée, 1995; Reibel et al., 1996).

Toxicity Reduction/ Toxicity Identification Evaluation (TR/TIE)

A more systematic way to address the effluent toxicity issue is to conduct formal TR/TIE investigations. The U.S. EPA has issued guidelines (Mount, 1988; Mount and Anderson-Carnahan, 1988a, 1988b; Fava et al., 1989) on how this may be performed. Essentially, the toxicity reduction evaluation (TRE) involves the following steps. Initially, the relevant data from effluent monitoring tests (e.g., BOD, toxicity tests), records of process circumstances (e.g., chemical usage) and evaluation of operating conditions (e.g., maintenance practices, consistency of operation) that may affect effluent quality are reviewed. These are the steps that are best undertaken by mill staff. If this does not lead to appropriate answers, then a systematic toxicity identification evaluation (TIE) procedure, including chemical analyses and toxicity testing, is undertaken. This is followed by a toxicity source evaluation (TSE) to track the agents identified from the TIE to a particular source within the plant. Toxicity treatability evaluation (TTE) and follow-up monitoring completes the task. The application of this approach for pulp and paper mill effluents has been summarized by Fein et al. (1994), Riebel and Lavallée (1995) and Riebel et al.

(1996). Examples of work investigating the source of toxicity within mills (Tomlinson, 1980; Naish et al., 1983; Scroggins, 1986) and TTE (Mueller et al., 1977; Schnell et al., 1990; Fein et al., 1993; Wong, 1994; Dufresne et al., 1995) type studies with mill effluents have also been published.

The TR/TIE methodology was intended mainly for helping industrial facilities design a strategy for achieving regulatory toxicity compliance (Fava et al., 1989). However, in the case of pulp and paper mills that already have secondary effluent treatment, the means of dealing with toxicity has been determined (e.g., aerated lagoon, activated sludge) and has been operating effectively for many years. What is needed is a methodology for elucidating the cause of the occasional toxicity breakthroughs. Presently, it is not known with certainty if the unmodified TR/TIE approach is suitable for this purpose. The task is particularly difficult when the toxicity is only marginal (e.g., 60% mortality at 100% concentration and no mortality at any of the lower concentrations tested) or when it is experienced intermittently (Orr, 1993; Reibel and Lavalée, 1995; Reibel et al., 1996). In the latter case, the difficulty is partly due to the time it takes to complete the toxicity tests and obtain the results. By the time it is ascertained that the effluent failed the acute lethal toxicity criterion, the final effluent might no longer be toxic and it would not be possible to identify the causes of toxicity.

The TIE component of the TR/TIE procedure was developed by the U.S. EPA to provide a systematic approach for identifying effluent components responsible for causing toxicity (Mount, 1988; Mount and Anderson-Carnahan, 1988a, 1988b). The approach involves effluent manipulation by various chemical and mechanical treatments in combination with toxicity testing. Typical effluent manipulations include aeration, pH adjustment, filtration, oxidation, chelation and solid phase extraction. While the TIE protocols are standardized to be applicable for many different types of effluents (e.g., industrial, municipal), customising the protocols for particular situations is encouraged (Orr, 1993; Reibel and Lavalée, 1995; Reibel et al., 1996).

The success of the TIE approach in pinpointing the causes of toxicity in a variety of different effluents, such as effluents from a chemical plant, publicly owned treatment works, a textile factory and municipalities have been well documented (Kop et al., 1981; Amato et al., 1992; Wells et al., 1994; Bailey et al., 1995). Literature references for the successful application of the TIE method to pulp and paper mill effluents have been more scarce. The available publications indicate that the method was successful in identifying biocides, polymers (Fein et al., 1994), ammonia (Reibel and Lavalée, 1995; Reibel et al., 1996) and cyanide (Zuercher, 1989) as causes of effluent acute lethal toxicity. Metals such as Zn and Cu were also found by this approach to be the agents causing chronic toxicity of a BCTMP effluent (Goudey et al. 1993).

One major difficulty in the application of the TIE procedure for solving problems of acute lethal toxicity of mill effluents to rainbow trout, the regulatory criterion in Canada, is the volume of effluent and the time required to conduct the toxicity tests. One way to deal with this problem would be to conduct tests with trout in 0.5 or 1 L solutions. Such non-conventional tests with trout may give a rough approximation of regulatory test results. Another way of handling the issue may be the use of surrogate test organisms. These surrogate species can include *D. magna* (in fact a test with this species is incorporated into the regulations for just such a purpose), *Ceriodaphnia* (another type of water flea), fathead minnow (*Pimephelas promelas*) larvae and the *Photobacterium* used in the Microtox® test. All of these species are much smaller than trout, require less exposure time and hence reduce the effluent volume, laboratory space requirements and costs for conducting the toxicity tests. In addition, Riebel et al. (1996), list a whole series of other types of microtests which are available for evaluating effluent toxicity.

While the use of surrogate species is an appealing option, and several authors (Fein et al., 1993, 1994; Firth and Backman, 1990) have shown that in some cases the Microtox® test could be used to identify mill effluent toxicity to *D. magna* and rainbow trout, caution is nevertheless advised. When the causes of toxicity to one species, for example rainbow trout, is sought, it may not always be possible to use

surrogate species for the TIE work since the various species may have different sensitivities to the toxicants present. As noted from our survey, approximately 30% of the time the effluent was toxic to rainbow trout but not to *D. magna*. Similarly, previous publications have reported substantial differences in the sensitivity of different organisms to pulp and paper mill effluent (Kovacs and Ferguson, 1990; Kovacs and Megrew, 1996). One study (Blaise et al., 1987) found that trout, algae and Microtox® tests could distinguish between non-toxic and toxic effluents with equal ability. The latter two tests were nevertheless somewhat more sensitive when the effluent toxicity thresholds were less than 100%. Therefore, if the use of surrogate species becomes crucial for reducing costs and speeding up the progress of the work, preliminary work establishing the sensitivity of the species to a particular effluent will be necessary.

Clearly, in the case of pulp and paper mill effluents, experience with the TIE methodology has not been extensive. It is not readily apparent that the TIE approach can be successful in all cases, especially when an effluent exhibits only marginal toxicity (Reibel et al., 1996). In these cases toxicity can be lost during the various manipulations. Further research into the applicability of the TIE protocols for pulp and paper mill effluents as well as the possibility of approaches specific for this industry may be worthwhile undertakings.

SUMMARY

In 1994-95, a survey about the acute lethal toxicity of effluents discharged by pulp and paper mills in Canada was undertaken. Of the 74 mills which responded to a questionnaire, 52 were operating secondary treatment facilities and 22 utilized primary treatment or other options. Approximately 35% of the mills with secondary-treated effluent experienced anywhere from one to greater than four episodes of acute lethal toxicity in a two year period. Of these mills, half could attribute the toxicity to known factors.

Based on the results of our survey and information in previous publications, properly designed secondary (aerobic) treatment facilities can eliminate the acute lethal toxicity of pulp and paper mill effluents during normal mill operating conditions. As such, ensuring tight operating conditions, minimizing spills and good housekeeping practises are the best means of reducing toxicity incidents. Nevertheless, if toxicity breakthroughs occur, steps can be taken by mill staff to identify the causative factors and thus help rectify the situation. These steps include examining the mill's record of operating conditions and ensuring appropriate toxicity testing conditions.

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TOXICITY IDENTIFICATION AND EVALUATION FOR THE PULP AND PAPER INDUSTRY. II. AN APPROACH BASED ON FISH STRESS SYMPTOMS

B.I. O'Connor[1], T.G. Kovacs[1], J.S. Gibbons[1], A.P. Strang[2] and H. Bailey[3]. [1]Pulp and Paper Research Institute of Canada, Pointe-Claire, QG; [2]Howe Sound Pulp and Paper Ltd., Port Mellon, BC; [3]EVS Environment Consultants, North Vancouver, BC.

During acute toxicity tests, rainbow trout exposed to effluent from an integrated bleached kraft, TMP and

Newsprint mill with a UNOX secondary-treatment system were found to exhibit signs of stress, including severely agitated behaviour followed by temporary immobilization, during the first hours of exposure. While the final effluent was generally not acutely lethal at full strength, it was important to determine the cause of the stress to ensure that the mill's effluent would always be in regulatory compliance. A series of experiments, combining chemical analyses with observations of fish symptoms, led to the identification of the causative agent.

A TOXICITY REDUCTION AND IDENTIFICATION EVALUATION OF A CHEMICAL PLANT WASTEWATER

A.P. Masters, A.G. Verbeek, M.A. Brown and N.K. Singh. CHEMEX Labs Alberta Inc., Edmonton, AB.

A final effluent sampled in 1995 from a chemical plant in Alberta, Canada, failed the trout test within 24 h and produced an eventual 96 h LC_{50} of 57%. The plant immediately stopped flow to the river and a Toxicity Reduction Evaluation was initiated. A second sample was immediately taken and the toxicity had declined; the sample failed the trout test within 24 h with an eventual 96 h LC_{50} of 73%. Four days after the initial failure, a third sample of final effluent was taken and this passed the 96 h trout test. Samples were taken from various in-plant streams and two were identified as the major potential sources of the toxicity; incinerator scrubber water and stripper's wastewater. A focused Toxicity Identification Evaluation was initiated on these waste streams. Removal of chlorine and subsequent spiking of chlorine to sodium thiosulphate treated incinerator water confirmed that hypochlorite was the cause of the majority of the toxicity of the incinerator water. Removal of chlorine and ammonia and subsequent spiking of the treated stripper's wastewater confirmed that ammonia was the cause of the majority of the toxicity in stripper's wastewater. The toxicity of this chemical plant's final effluent was likely a result of an interaction between the toxic components in these two streams.

TOXICITY IDENTIFICATION AND EVALUATION OF MFO-INDUCING FRACTIONS OF EFFLUENT FROM AN OIL REFINERY IN ONTARIO

J.L. Parrott, B.F. Scott and J.P. Sherry. Environment Canada, National Water Research Institute, Burlington, ON.

Final effluents collected from four Ontario oil refineries were found to induce mixed function oxygenase (MFO) in small rainbow trout exposed for 4 d in the lab. Toxicity Identification and Evaluation (TIE) was initiated with one effluent, using MFO activity in whole fish or in fish or rat liver cell lines as the biological endpoint. This effluent also caused mortality in larval fathead minnows. Whole effluent acidified and extracted with dichloromethane (DCM) gave a similar dose-response curve as unextracted whole effluent. Non polar (hexane) and slightly polar (DCM/hexane) extracts of effluent did not induce MFO in fish, while polar (methanol) extract induced MFO to levels as high as extracts of whole effluent. Further splitting of the methanol fraction into less polar (DCM), mid-polar (DCM/isopropanol) and more polar (methanol) extracts yielded induction in the mid-polar, DCM/isopropanol fraction. Rat and fish liver cell lines exposed to fractions showed similar results, though the distinction was not as dramatic as the whole fish tests. Rat and fish cell lines tended to show elevated MFO in two or more fractions, though the patterns of most to least-inducing fraction was similar to whole fish. The results are unusual and unexpected, in that most traditional MFO inducing compounds would be found in the non-polar or slightly polar fractions, as they are usually lipophilic neutral organic chemicals. Investigations into the nature and identity of the refinery effluent inducer(s) are continuing.

MOLECULAR TOXICOLOGY ASSOCIATED WITH NATURAL NAPHTHENIC ACIDS ISOLATED FROM SYNCRUDE TAILINGS POND WATER AND COMMERCIALLY AVAILABLE NAPTHENIC ACIDS

D.A. Birkholz[1], R.E. Rogers [2] and M. MacKinnon[3]. [1]Enviro-Test Laboratories, Edmonton, AB; [2]Toxcon Inc., Edmonton, AB; [3]Syncrude Research, Edmonton, AB.

Toxicology, as used for predicting public health and environmental risks, is the study of how specific chemicals cause injury to living cells and whole organisms. Molecular toxicology can be used to predict cell damage as a consequence of exposure to chemicals because all species have the same basic structural makeup of deoxyribonucleic acid (DNA), the carrier of genetic information in cells. Toxicological responses in organisms as a results of chemical exposure may be linked to damaging cell membrane integrity, protein packaging, respiration, osmotic conditions, DNA damage and universal cellular stress.

The objective of this study was to subject the following samples to cytotoxicity studies using *Escherichia coli* and the Pro-Tox® cellular assay [1] Fresh Syncrude Tailings Water from the Mildred Lake Settling Basin (SynTPW), [2] low molecular weight napthenic acids isolated from tailings water (TPWEXT), and [3] commercially available napthenic acids (NAP P&B). Induction of cellular genes was revealed for all samples tested. In all cases, the largest response was for osmY and this response correlated well with observed cytotoxicity (SynTPW - 1.8-fold, EC_{50} >50 mg/L; TPWEXT - 4.0-fold, EC_{50} = 45 mg/L, and NAP P&B - 4.8fold, EC_{50} = 35 mg/L). A similar trend is observed for induction with micF (SynTPW - 1.6-fold, TPWEXT - 3.0-fold, and NAP P&B - 3.3 fold). It would appear that membrane shutdown is the main mechanism for cytotoxicity associated with these samples. Such a response is observed when the Pro-Tox® assay is applied to surfactants. Since napthenic acids have surfactant properties, such a response is not unexpected. The higher toxic response observed for commercial naphthenic acids (NAP P&B) may be explained by the fact that napthenic acids have at least four structural forms. Some of these forms may have greater surfactant properties and hence give rise to greater micF and osmY responses. Commercial formulation may contain higher levels of these forms than those found in natural tailings water (TPWEXT).

Minor DNA inductions (1.4-1.7 fold) were observed. Correlations with other cellular assays suggest that induction levels of 20-30 fold are required with recA, unuDC, dinD, and nfo before DNA damage occurs. These genes are all involved with the SOS DNA repair system in the cell. The inductions reported in this study for DNA damage are minor and would not constitute a threat of cellular damage through this mechanism. Based on these studies a NOEL and LOEL for gene induction of 1.0 mg/L and 10.0 mg/L, respectively, were determined for Fresh Syncrude Tailings Water from the Mildred Lake Settling Basin and commercial napthenic acids.

REGULATORY HARMONIZATION

SESSION CHAIR: T. WATSON

ENVIRONMENT CANADA IMPLEMENTATION OF THE FEDERAL TOXIC SUBSTANCES MANAGEMENT POLICY

R. Chénier, C. Fortin, C.F.R. Morcos, D. Campbell and G. Owen. Environment Canada, Toxics Pollution Prevention Directorate, Hull, QC.

Adoption of the federal government Toxic Substances Management Policy was announced in June 1995. The policy provides a science-based framework for the management of toxic substances. The key management objectives in the policy are [1] virtual elimination from the environment of anthropogenic toxic substances that are persistent and bioaccumulative (referred to as Track 1

substances), and [2] management of other toxic substances and substances of concern, throughout their life cycles, to prevent or minimize their release into the environment (referred to as Track 2 substances). This presentation will outline key initiatives undertaken by Environment Canada in implementing the policy. Examples include domestic initiatives such as the Strategic Options Process, the Federal/Provincial Task Force on Dioxins and Furans, the preparation of scientific justifications for candidate Track 1 substances, and international initiatives undertaken under the UN-ECE LRTAP convention, the UNEP and NAFTA CEC.

CCME SYSTEM FOR CONTAMINATED SITE ASSESSMENT AND REMEDIATION

G.E. Nason[1] and C. Gaudet[2]. [1]Alberta Environmental Protection. Lethbridge, AB; [2]Environment Canada, Ottawa, ON.

The Canadian Council of Ministers of the Environment (CCME) is a federal-provincial cooperative body that identifies and addresses high priority environmental concerns through development of strategic options, cost-shared programs, and management tools including guidelines and codes of practice. In 1990 CCME launched the National Contaminated Sites Remediation Program, which had the goals of [1] remediation of high risk orphan contaminated sites, [2] development and demonstration of innovative remediation technologies, and [3] presentation or development of scientific tools that would ensure effective and consistent approach to the identification, assessment and remediation of contaminated sites. This presentation provides an overview of activities and products addressing the final goal. Initial efforts were directed to development of a framework for contaminated sites management that embraces all general steps from site identification to attainment of closure conditions. Once this framework was established, highest priority was given to development of a classification tool and generic numerical guidelines that would, respectively, direct efforts to the areas of greatest concern (CCME National Classification System for Contaminated Sites, 1992) and establish assessment and remediation benchmarks for sites requiring immediate attention (CCME Interim Environmental Quality Criteria for Contaminated Sites (1991). Subsequently, most effort has gone into development of [1] guidance for site-specific risk assessment, [2] scientifically-defensible effects-based numerical guidelines for soil, and [3] guidance on development of remediation objectives from guidelines or site-specific risk assessment. Currently, guidelines are being provided for four land use categories that are both meaningful to average Canadians and important to regulators and planners. These are agriculture, residential/parkland, commercial and industrial. Each land use category has an associated exposure scenario and set of protection goals. Because numerical guidelines for soil under these land uses are based on transparent, scientifically-based pathway and exposure analyses, it is possible in some cases to adjust guideline values to take account of site-specific differences in influential parameters. This presentation will review the assessment/remediation framework and examine options for development of remediation objectives as driven by information collected in a phased investigative strategy.

"JUST HOW TOXIC IS HIGHLY TOXIC?" *OR* THE IMPORTANCE OF HARMONIZATION IN THE CLASSIFICATION OF TOXICITY

J. Schroeder[1], G. Gilron[1] and J. Cerf[2]. [1]BEAK Consultants Ltd., Brampton, ON; [2]Union Carbide Canada Inc., Anjou, QC.

Standardized testing protocols and modern statistics make it simple for researchers to quantify toxic effects. The LC_{50}, for example, is a familiar and well-accepted endpoint for estimating the lethal effect of a chemical product or effluent. But how do we use an LC_{50}, or any other endpoint, to define or qualify of the toxic effect? We conducted a literature review of toxicity/chemical hazard classification

schemes employed by international, federal and provincial. Quantitative endpoints are converted into phrases such as 'highly toxic' or 'environmentally acceptable' that serve as management tools in compliance and registration regulations, however, because these phrases are often based on one or a few endpoints, they may not account for all relevant toxic effects or the degree of the associated ecological risk. It is important to consider the likely environmental receptors in defining which measured toxic responses are relevant to the classification. Moreover, standards should be set to form the foundation of consistent, comparable classification schemes. We developed and present here a rationale for a standardized classification scheme based on ecologically relevant data with the purpose of harmonizing the interpretation of toxicity and creating definitions that are meaningful and representative of scientific data.

DEVELOPMENT OF THE UNITED STATES DEPARTMENT OF DEFENSE ECOLOGICAL RISK SCREENING PROCESS

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ABSTRACT

The Rational National Standards Initiative (RNSI) is a program initiated by the U.S. Air Force Air Combat Command (ACC) to develop risk-based screening procedures for future land use. In conjunction with the ACC and the U.S. Army Corps of Engineers, Radian International has developed the risk-based screening protocol for human receptors that is currently being used at Department of Defence (DoD) facilities throughout the U.S. Currently, the Navy Environmental Health Center has joined the project team in an effort to develop a draft ecological screening procedure that will soon be finalized for use at DoD facilities. The technical approach utilizes a suite of endpoint species and food chain models that represent primary ecological communities at DoD facilities. The screening process develops ecological screening values for soil, surface water and sediment exposures. For specific sites, the screening results will be used to make 'no further action' decisions, guide additional investigations and/or support preparation of detailed ecological risk assessments. While this screening process is being developed for DoD facilities, it also has applicability to contaminated sites outside of the DoD.

INTRODUCTION

The Rational National Standards Initiative (RNSI) is a program initiated by the U.S. Air Force Air Combat Command (ACC) to develop a risk management tool for future land use. The motivation of this program is to focus facility funding on sites where there are realistic risks to either human health or the environment. In conjunction with ACC and the U.S. Army Corps of Engineers (Omaha, NE), Radian International initially developed RNSI to provide quantitative risk evaluations for human health and qualitative risk evaluations for ecosystems. However, as the project progressed, it became apparent that a quantitative screening risk evaluation also would be necessary to evaluate potential ecological impacts. Currently, the Navy Environmental Health Center (Norfolk, VA) has joined the project team to develop the ecological screening procedure that will soon be finalized for use at Department of Defense facilities. This paper focuses on the methodology that has been developed for ecological risk screening assessments.

The RNSI program now provides site managers and stakeholders with a human health and ecological risk management tool that has four primary objectives. First, risk-based screening levels are provided for affected media based on expected future land use and ecological habitat. Second, remedial technologies and costs that may be required to clean up to the risk-based screening levels are provided as point of departure for planning purposes. Third, this information is provided in a concise

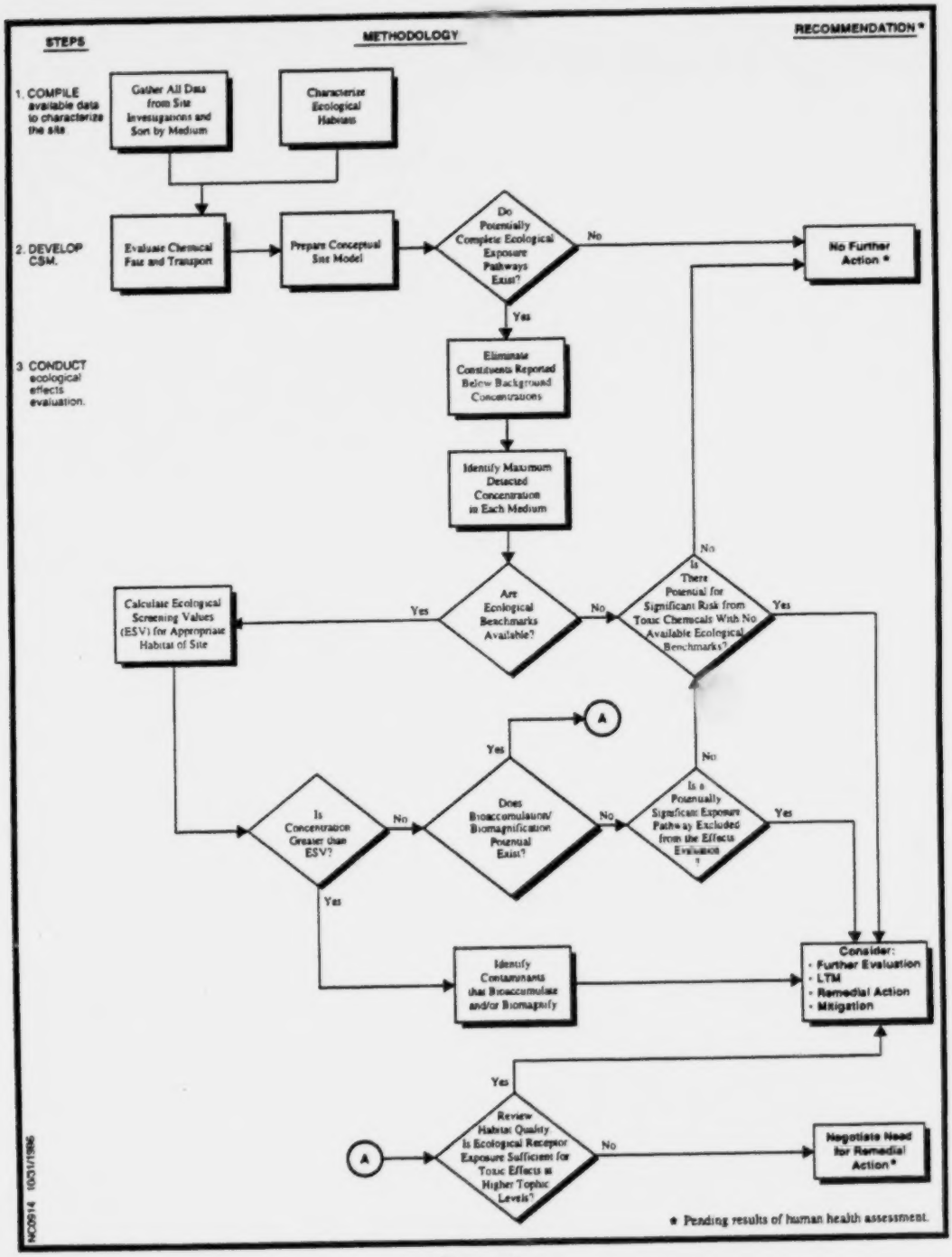


Figure 1.
Screening Level Ecological Assessment Methodology

document and is intended to be used to make 'no further action' decisions, guide additional investigations, and/or support preparation of more detailed risk analyses. Finally, the risk screening levels may be used as actual clean-up criteria, if managers and stakeholders agree that it is cost effective to clean up to the screening levels rather than conduct further investigations and risk analyses to derive more precise risk-based clean-up goals.

Ecological Risk Assessments

Characterization of risk to an ecological component is typically performed through evaluation of assessment endpoints. An assessment endpoint is an explicit expression of the ecological component considered to be at risk. Generally, assessment endpoints have associated target species, or assessment endpoint species, that are primary receptors at a site. A measurement endpoint is a measurable response to a stressor (e.g., a chemical contaminant) that is related to the assessment endpoint (Suter, 1993). Measurement endpoints are based on laboratory toxicity studies that allow quantification of specific potential effects to assessment endpoint species.

There are several levels of ecological risk assessment ranging from formal to screening level assessments. The amount of detail required for the various levels of an ecological risk assessment is dependent on the amount of uncertainty that is considered acceptable for the study area or site. Formal ecological risk assessments typically consist of a problem formulation with testable hypotheses of potential ecological effects, detailed habitat descriptions, assessment and measurement endpoints and site specific assessment endpoint species, analysis of exposure which includes food-chain effects and multiple exposure pathways, and a risk characterization and uncertainty analysis with recommendations for site specific action. Formal ecological risk assessments are typically supported with more detailed site-specific data for site flora and fauna than are screening level assessments.

Screening level ecological risk assessments consist of a preliminary problem formulation which includes general habitat descriptions and conceptual site exposure models that are used to generate hypotheses of potential ecological effects. The screening assessment then analyzes potential effects using a small number of screening target species (assessment endpoint species) and simple exposure pathways. The final step is a risk characterization that focuses on effects to individuals which also are qualitatively extrapolated to populations. The RNSI ecological screening is a screening assessment that uses the ecological screening methodology contained in the U.S. Environmental Protection Agency's Framework for Ecological Risk Assessment (EPA, 1992). As a result of the RNSI ecological screening, recommendations may be made for [1] no further action, [2] remediation, [3] mitigation, [4] monitoring, [5] further site investigations, and/or [6] performance of a formal ecological risk assessment.

The uncertainty associated with estimates of potential risk in screening assessments is relatively high. However, very conservative exposure assumptions are utilized to develop chemical-specific screening values in order to minimize the probability of concluding that a contaminant does not pose potential risk when it actually does pose risk. In statistical terms, if the null hypothesis is that a site is below acceptable risk levels, the process of estimating potential risk should be performed in a manner that will reduce Type II error, or accepting the null hypothesis when it is actually false.

RNSI Ecological Screening Assessment Approach

Department of Defense facilities occur over a wide area of the U.S., and thus, are not confined to one ecological region. The scope of our work has been to develop a common evaluation process that would screen out those areas that are not posing an ecological risk and identify those sites that have the potential to pose a risk. In most cases, analytical data are available for soils, groundwater, and surface water and sediments if applicable. A flowchart depicting the screening process is presented in Fig. 1.

Given our goal of finding a common way to evaluate sites in various ecological regions across the U.S., the first step was to identify general habitat types that were commonly found at the nationwide facilities. We identified these as grasslands, forests, deserts, wetlands, and surface water bodies. Next, we

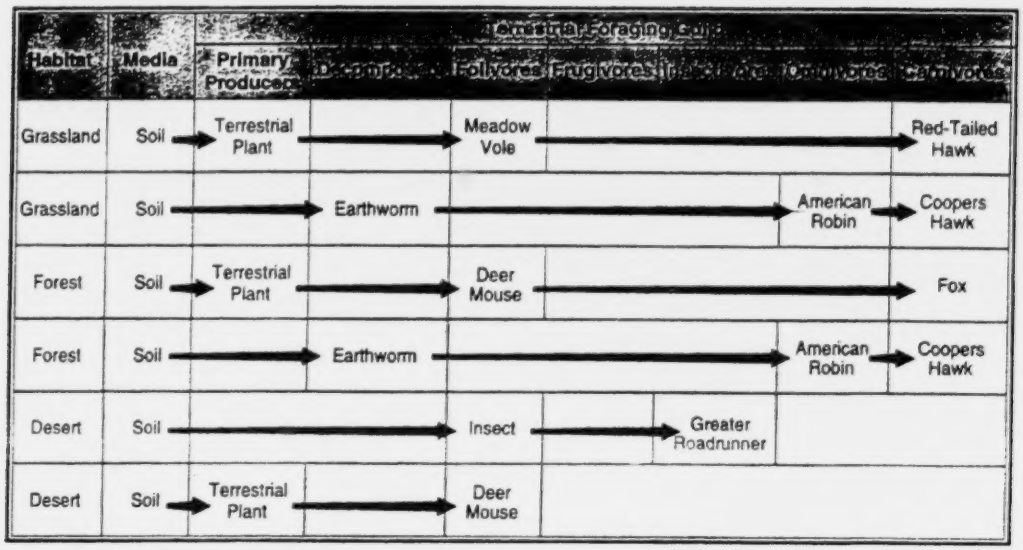


Fig. 2. RNSI terrestrial assessment endpoint species and trophic pathways.

selected common assessment endpoint species applicable for each of these habitats. These are shown in Fig. 2 for terrestrial fauna. For aquatic systems, specific assessment endpoint species were not selected because available water quality and sediment criteria may be applied to a wide variety of aquatic organisms. Similarly, plant risk screening benchmarks developed by Will and Suter (1995) may be applied to many terrestrial flora.

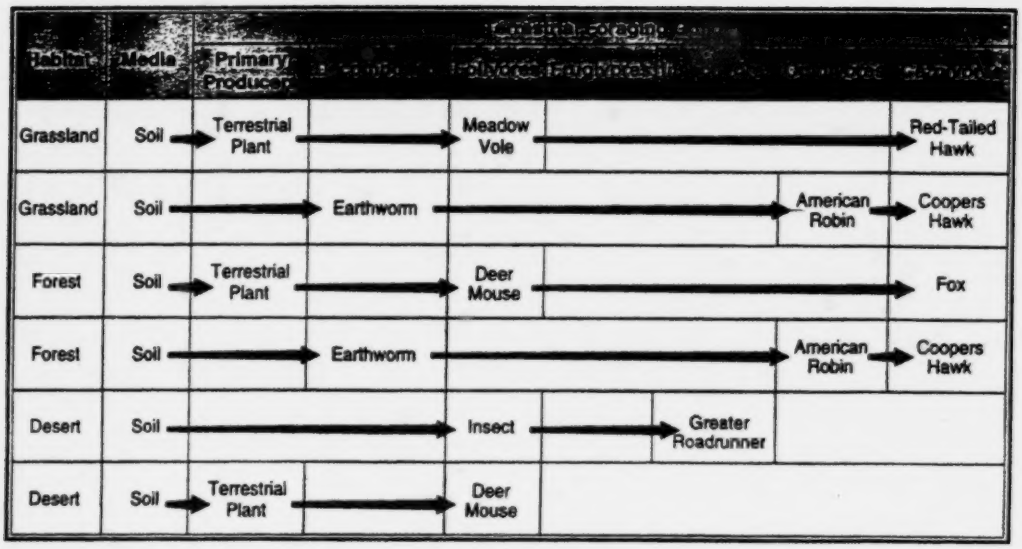
We then developed algorithms to calculate ecological screening values representing exposures to soil, surface water, or sediment. These algorithms utilize conservative, default exposure assumptions for quantifying risk. The target risk was set as an ecological hazard quotient of 1.0.

Data Compilation

For a given site, available data is compiled, the site is visually observed and characterized for ecological impacts. This information is documented in a summary-type format with supporting data appendices. The summary documentation easily can be discussed with parties interested in the evaluation.

The data compilation step includes gathering site-specific biological survey data and chemical data that has been obtained from sampling programs conducted at the site, including any available background chemical data. Background data may be naturally occurring inorganic concentrations and/or ambient background. Anthropogenic chemistry that is generally widespread because of urbanization/industrialization is included in ambient background.

Ecological habitats are characterized through visual observations during site visits and literature searches. The primary ecological community types and the dominant species within these communities are documented. For most terrestrial and many wetland systems, the characterization of dominant species will focus on vegetation. By knowing the dominant vegetation types, dominant fauna likely to inhabit the site may be identified. A notable component of the habitat characterization is evaluating the potential for protected species' use of the site. A checklist has been prepared for use in compiling ecological community data.



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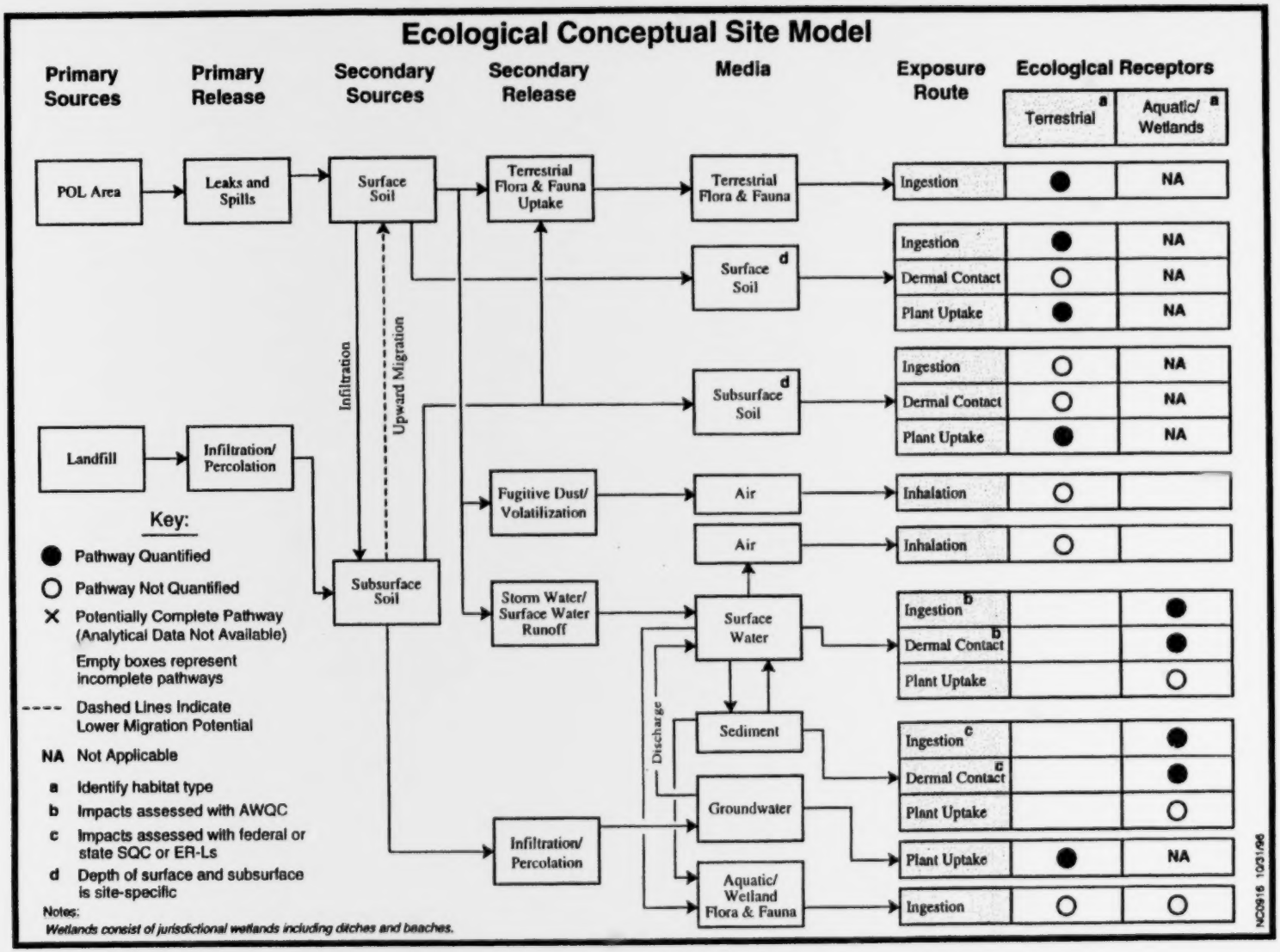

Figure 3. Generic Basewide Conceptual Site Model

Table 1

Ecological Screening Value Algorithm for the Red-Tailed Hawk
Trophic Pathway: Soil → Plant → Meadow Vole → Red-Tailed Hawk

$$ESV_i \text{ (mg/kg)} = \frac{(EHQ)\ (BW_H)\ (NOAEL_H)}{HR_H\left[S_H + FI_H * BAF_{MV}\left[\frac{(UP_{i\,plants} \times FI_{MV}) + S_{MV}}{FI_{MV} + S_{MV}}\right]\right]}$$

Exposure Variables	Definitions	Default Values
ESV_i	Ecological Screening Value for Chemical i	mg/kg
EHQ	Ecological Hazard Quotient	1 (unitless)
BW_H	Body Weight, Red-tailed Hawk	1.028[a] kg
$NOAEL_H$	No Observed Adverse Effects Level, Red-tailed Hawk,	Chemical-specific mg/kg/day
HR_H	Home Range Factor (site area/species home range), Red-tailed Hawk	Site area/148[b,e] (unitless)
S_H	Soil Ingestion Rate, Red-tailed Hawk	0.003[c] kg/day
FI_H	Food Ingestion Rate, Red-tailed Hawk	0.055[b,f] kg/day
$UP_{i\,plants}$	Plant uptake factor for Chemical i	Chemical-specific (unitless) [g]
FI_{MV}	Food Ingestion Rate, Meadow Vole (kg/day)	0.0049[b] kg/day
S_{MV}	Soil Ingestion Rate, Meadow Vole (kg/day)	0.0001176[d] kg/day
BAF_{MV}	Bioaccumulation Factor, Meadow Vole	Chemical-specific (unitless) [h]

[a] Dunning, 1993.
[b] EPA, 1993.
[c] No literature value available, assume 5% of food ingestion rate.
[d] Beyer et al., 1994.
[e] Home range factor is dependent on site size.
[f] Nagy, 1987.
[g] Baes it al., 1984; Briggs et al., 1983; Travis and Arms, 1988.
[h] In the absence of a BAF_{MV}, assume $BAF_{MV} = 1.0$.

Identification of Exposure Pathways

The information acquired in the site characterization is used to produce a conceptual site exposure model. This model provides the primary exposure pathways of concern for each site's future land use. An example conceptual site model is presented in Fig. 3.

Ecological Effects Evaluation

If the conceptual site model indicates that exposure is possible, ecological screening values are calculated using available toxicity values (measurement endpoints) from the literature. An example ecological screening value calculation is provided in Table 1. Measurement endpoints for terrestrial pathways are chronic toxicity studies quantifying the no-observed-adverse-effect-level (NOAEL) for mortality or reproductive effects. The NOAEL data are taken from the available literature for those species used as assessment endpoint species. If toxicity data are not available for these species, toxicity data are used for surrogate species that are similar physiologically and in life history patterns to the assessment endpoint species. Use of surrogates is, in practice, often required in performing ecological risk evaluations. This adds uncertainty to the evaluation results and is an unfortunate fact resulting from the lack of toxicity research for many ecological receptors. However, measurement endpoint surrogate species are not used that are taxonomically dissimilar from assessment endpoint species beyond the level of Order. Depending on the level of taxonomic and physiological dissimilarity between surrogate and assessment endpoint species, adjusting toxicity values for difference in body weight, in accordance with Opresko et al. (1995) may be appropriate.

If NOAEL data are unavailable, chronic lowest-observed-adverse-effect-level (LOAEL) data are used after reduction by an uncertainty factor of 0.1. Additionally, when NOAEL or LOAEL data are from acute rather than chronic studies, the data are reduced by a factor of 0.1 in order to be protective of potential chronic effects from long-term exposures. Studies are classified as chronic or acute on the basis of their duration and test organism. Studies are considered to provide chronic toxicity data if conducted for a minimum of 90 d in mammals, 28 d in birds, or 7 d in invertebrates. Shorter studies are considered acute.

The maximum detected chemical concentrations at the site are compared to the calculated ecological screening values. For terrestrial systems, the calculated ecological screening values are the highest soil concentrations that are expected to result in no adverse effects to terrestrial assessment endpoint species. For aquatic systems, appropriate Federal, state, or regional water quality criteria and sediment benchmarks are used as the ecological screening values.

In addition to comparisons to the ecological screening values, the bioaccumulation and biomagnification potential of contaminants are evaluated to determine the potential for effects to upper trophic levels that may not be addressed by the screening algorithms. For example, in soils bioavailability is affected by whether or not the chemical is hydrophilic or hydrophobic and the potential for surface runoff and leaching. Additionally, soil physical characteristics, such as organic matter content, affect bioavailability and environmental fate.

Recommendations are provided to assist the manager and stakeholders in planning site activities. These recommendations can include no further action, further evaluation, long-term monitoring, remedial action such as capping or removal of contaminants, or mitigation such as creation of a similar habitat off-site to compensate for the habitat loss on-site. Recommendations are based on those chemicals detected above ecological screening values and are habitat and media specific. The EPA Remediation Technologies Screening Matrix and Reference Guide (EPA and USAF software, 1994) is used to help select appropriate remedial technologies. This software was developed by the U.S. Air Force and the U.S. EPA, with extensive input from professionals in the field to provide guidance for the selection of technologies to clean up hazardous waste sites. Remedial technologies are chosen which represent the most cost-effective technologies that have been proven effective in removing contamination at sites. In addition, the RNSI documentation provides comments regarding the appropriateness of remediating

a site as it relates to potential ecological hazards posed by remedial activities.

ACKNOWLEDGEMENTS

The RNSI project was initially developed and executed for the U.S. Air Force, Air Combat Command under the direction of Ms. Terrie Warren through the Omaha District Corps of Engineers with Mr. Larry Janis as the technical manager and Ms. Anita Meyer as the project industrial hygienist. Ms. Vera Wang and Ms. Yvonne Walker with the Navy Environmental Health Center also have contributed in the development of RNSI. Radian International, under the direction of Ms. Kathleen Alsup as the project manager and Dr. Jeff Beacham as technical manager, has prepared the documentation and assisted in the development of the prototype documents. Ms. Margaret Roy of Radian also has been heavily involved in the development of the RNSI ecological screening methodology.

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KEY RECOMMENDATIONS OF AQUAMIN

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The Final Report of the Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN) was presented to Environment Canada in July 1996 for consideration in the regulatory review process. The report was prepared by representatives of government, industry, Aboriginal groups, and environmental non-government organizations. The key recommendation, supported by all stakeholders involved in the preparation of the report, is that a cooperative national environmental protection framework be implemented, that would include three components [1] the Metal Mining Liquid Effluent Regulations (MMLER), revised as recommended in the Final Report, would continue to ensure a consistent minimum

quality of effluent being discharged to aquatic ecosystems, [2] the establishment, if necessary, of site-specific requirements: more stringent requirements to ensure adequate protection of some aquatic ecosystems, and [3] the implementation, as recommended in the report, of a national environmental effects monitoring (EEM) program for metal mining, would serve as a feedback loop, providing information to decision-makers and the public regarding the effectiveness of both environmental protection measures and long-term regulatory strategies.

Recommendations emphasize the need to involve all stakeholders, nationally, regionally and locally. Further, the report emphasizes the need for a greater degree of cooperation between federal and provincial regulatory agencies, to ensure effective and efficient protection of the environment. With regards to the MMLER, the report recommends that efforts be made to increase compatibility and reduce duplication between regulatory agencies and to reduce compliance costs. In cases where other jurisdictions have legally enforceable requirements, it is recommended that the federal government reach delivery agreements with the other jurisdictions to establish single reporting mechanisms. With regards to EEM, the report recommends that all stakeholders be involved in the study design phase, and that designs be tailored to each site. This will ensure that a national requirement for EEM will enhance and complement, rather than duplicate, existing monitoring requirements.

ENVIRONMENTAL EFFECTS MONITORING (EEM) - PULP AND PAPER

Session chair: B. Steinback

PULP AND PAPER ENVIRONMENTAL EFFECTS MONITORING PROGRAM: CYCLE 1

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The Environmental Effects Monitoring (EEM) program for the pulp and paper industry is a national program to assess, on an iterative cycle approach, the effectiveness of the Pulp and Paper Effluent Regulations of the Fisheries Act by monitoring the effects of effluents on the aquatic environment. The evaluation examines the magnitude, extent and trends of the effects in the receiving environment of the pulp and paper mills effluents. The EEM program provides for a 'baseline' study (Cycle 1) where the methodology of the approach is to be evaluated. This cycle was conceived to provide the information and experience upon which the next cycle would be improved. In situations where the technical and the scientific elements of the approach presented difficulties, modifications or alternatives would be sought to ensure the soundness of the scientific foundation of the subsequent cycles of the program. Cycle 1 of the EEM program has been completed and a government/industry joint review of the results of this first cycle is nearly completed. The presentation will provide an overview of the Cycle 1 experience, of the specific issues being addressed as a result of problem identification and of the status of the EEM program.

EEM CYCLE 1: LESSONS LEARNED

G.P. Thomas and N. Munteanu. G3 Consulting Ltd., Richmond, BC.

Subsequent to the completion of Cycle 1 of the pulp and paper EEM investigations, evaluation by regulators, industry, and scientists was undertaken in BC. Discussions and recommendations forwarded by these groups on the successes and shortcomings of specific components and parameters used,

design approaches, interpretation and tools employed, and general design approaches has provided useful insight to the development of subsequent or related environmental initiatives. This paper provides a discussion of general recommendations forwarded by consultants, industry and government with respect to the National EEM programs and applications regionally.

A COMPARISON OF THE RESULTS OF CYCLE 1 MARINE EEM AQUATIC TOXICITY TESTING OF PULP AND PAPER MILL EFFLUENTS

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Marine toxicity tests were conducted quarterly under Canada's Environmental Effects Monitoring program. The toxicity tests included the 7-d *Champia parvula* test, the 7-d silverside (*Menidia beryllina*) test, and the echinoid sperm cell fertilization test. All three tests were performed on routinely, however, *Champia* exhibited an appreciably higher failure rate. The responses of the different species were not correlated. The echinoid test exhibited the greatest sensitivity, followed by *Menidia*, and *Champia*. In general, IC_{25} estimates were similar to the NOECs for *Menidia*, were greater than the NOECs for the echinoderms, and less than the NOECs for *Champia*. Due to sample variability and limited sample size, it was not possible to distinguish between different mill production or treatment processes with respect to toxicity. The results suggest that toxicity tests have a useful role in identifying toxicity in these effluents and that Toxicity Identification Evaluations should be conducted to identify the causes of toxicity so that effective treatment and/or source control measures can be implemented.

A COMPARISON OF THE RESULTS OF CYCLE 1 FRESHWATER EEM AQUATIC TOXICITY TESTING OF PULP AND PAPER MILL EFFLUENTS

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Freshwater short-term chronic toxicity tests were conducted quarterly under the Environmental Effects Monitoring program. The toxicity tests included the 7 d *Ceriodaphnia dubia* test for survival and reproduction, the 7 d rainbow trout (*Oncorhynchus mykiss*) embryo viability test, and the 72 h *Selenastrum capricornutum* algal growth inhibition test. All three tests were performed on a routine basis with few failures. The responses of the different species were generally not correlated. The algal test exhibited the greatest sensitivity, followed by the trout embryo and *Ceriodaphnia* tests. In general, IC_{25} estimates were greater than the no-observable effect concentrations. Due to variability between samples from each mill and small sample size, it was not possible to distinguish between different mill production and treatment processes with respect to their effect on toxicity. The results suggest that toxicity tests have a useful role in identifying toxicity in these effluents and that Toxicity Identification Evaluations should be conducted to identify the cause of toxicity so that effective treatment can be implemented.

SUMMARY OF SUBLETHAL TOXICITY TEST RESULTS FROM THE FIRST CYCLE OF THE PULP AND PAPER ENVIRONMENTAL EFFECTS MONITORING PROGRAM

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With the 1992 Amendments of the Pulp and Paper Liquid Regulations, a new element was introduced which required the pulp and paper industry to conduct an Environmental Effects Monitoring (EEM) program at mill locations across Canada to assess whether or not the national effluent regulation was protective of fish populations, fish habitat and human use of the fisheries resource. One of the specific monitoring requirements for the first cycle of the EEM program was the measurement of sublethal toxicity in samples of a mill's final treated effluent on four occasions throughout the year in which the program was conducted. During 1994 or 1995, a battery of three toxicity tests were used to assess potential sublethal effects on [1] early-life-stage development of fish, [2] reproduction of an invertebrate, and [3] growth of an aquatic plant. The choice of tests and species depended on whether the mill's effluent was discharging to a fresh or estuarine/marine receiving waterbody.

On April 1, 1996, 125 interpretative reports describing the results for the first cycle of EEM were submitted to the regional offices of Environment Canada. Regional staff handled the first major task which was to examine the sublethal toxicity results to ensure that method QA/QC requirements had been met and that the test results were valid. Following the QA/QC check, Regional staff extracted the sublethal toxicity data for the four sampling periods and submitted this information to staff of the Method Development and Application Section for compilation of the results on a national level and to facilitate discussion between government and industry experts on sublethal toxicity testing requirements for the second cycle of EEM. Although different types of endpoints were generated for each test (i.e. IC_{25}, IC_{50}, LC_{50}, and LOEC/NOEC/TEC), only IC_{25} results and calculated Predicted Minimum Toxic Concentrations (PMTCs) will be discussed. A summary of freshwater and marine sublethal test data from all Canadian pulp and paper effluent discharges will be presented with discussion focused on test sensitivity trends, comparability of results between freshwater and marine tests and variability of effluent toxicity.

HEALTH ASSESSMENT OF MOUNTAIN WHITEFISH (*Prosopium williamsoni*) FROM THE COLUMBIA RIVER IN JULY 1994

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ABSTRACT

In July, 1994, adult mountain whitefish, *Prosopium williamsoni*, were sampled from three locations near Castlegar, BC, at the Genelle reach of the Columbia River, downstream of a bleached kraft pulp mill; at the Beaver Creek reach of the Columbia river, downstream of a Pb/Zn smelter; and from a reference site on the Slocan River. This was the second in a series of three studies (in July of 1992, 1994 and 1996) designed to monitor anticipated improvements in fish health and chemical contamination following upgrading and process changes at the pulp mill and modernization of the smelter. Results of the 1994 study showed no evidence of reduced growth, energy storage, or reproduction in whitefish from the Genelle and Beaver Creek reaches of the Columbia River. However, mountain whitefish from the Columbia River had a higher incidence and severity of abnormalities when compared to the reference site, suggesting impaired health. Concentrations of polychlorinated dibenzodioxins (PCDD) and dibenzofurans (PCDF) in Columbia River whitefish muscle tissue have declined significantly since 1991, while polychlorinated biphenyl congeners (PCB) have remained stable. Mixed function oxidase (MFO) activities also exhibited significant reductions since 1991. Concentrations of metals in muscle tissue remained low at all sampling locations. Cu and Cd increased in liver tissue in fish caught below the smelter between 1992 and 1994. These metals continued to be significantly correlated to metallothionein (MT), suggesting active metal uptake at this reach only.

BACKGROUND

In the early 1990s a number of fish health and chemical contamination problems were documented in mountain whitefish, *Prosopium williamsoni*, sampled from the Columbia River near Castlegar, BC (e.g., Boyle et al., 1992; R.L. & L. Environmental Services Ltd., 1992). In response to these concerns, the Department of Fisheries and Oceans (DFO) initiated a five year study under the Green Plan Toxic Chemicals Program to examine the health status and chemical contamination of mountain whitefish from the Columbia River following the upgrading of the pulp mill in Castlegar, and modernization of the smelter/fertilizer complex in Trail, BC. Changes at the pulp mill included secondary treatment and 100% chlorine dioxide substitution, which were completed by June 1993. The ongoing abatement projects implemented at the smelter continue to reduce the loading of metals into the Columbia River.

The study consisted of three sampling events, in July of 1992, 1994, and 1996. Results of the first study in July 1992 (Nener et al., 1995) indicated that fish sampled from the Genelle reach of the Columbia River (below the pulp mill) exhibited consistently higher frequencies of abnormalities and significantly greater disease severity than fish sampled from the Beaver Creek reach of the Columbia River (below of the smelter), or the reference site on the Slocan River (Nener et al., 1995). The 1992 fish health assessment results were similar to results from a preliminary study conducted by DFO in January 1991, prior to the upgrading of the pulp mill (Boyle et al., 1992). PCDD, PCDF and PCB concentrations in muscle tissue were also higher at the two Columbia River sites than at the reference site in 1992. Muscle metal concentrations, however, were generally highest in fish from the reference site. This paper summarizes results of the second sampling program which took place in July 1994.

Fish Health Assessment Method

Adult mountain whitefish between 250 to 400 mm in length were collected between July 5, and July 14, 1994, from two reaches on the Columbia River: at Genelle, approximately 10 km below the pulp mill; and at Beaver Creek, about 12 km below the smelter. Both these reaches are located downstream of the Hugh Keenleyside Dam. Fish were also sampled from a reference site on the Slocan River, a tributary to the Columbia River. Fish were caught by electroshocking using a low voltage setting. Individual fish were examined using standard disease testing protocols (Department of Fisheries and Oceans, 1984).

A total of approximately 73 fish per site were analyzed for wet weight, fork length, condition factor, age, and disease survey, including gross external and internal examination, bacteriology, and histopathological examination of gill, liver, spleen, kidney and pyloric caeca/hindgut. A subset of these fish from each reach was analyzed for PCDD, PCDF, and PCB in muscle tissue (n=10), and MFO activities (n=15). Another subset of fish was analyzed for metals in muscle tissue (n=15) and MT (n=15). A separate set of 10 fish per reach were analyzed for parasitology.

A Cumulative Disease Severity (CDS) rating system was developed to help interpret the fish health significance of the disease survey results. The system rates the various types of abnormalities as to their potential impact on fish health. An abnormality such as a parasite cyst was rated light (1), the impact of causative organisms such as myxobacteria was considered to be moderate (2), and the presence of mycobacteria was considered more damaging, and was rated as severe (3). Normal condition was rated as (0). The total numerical value of all abnormalities found in one fish at a given location was used to indicate the CDS, or overall health status of each fish.

Key Findings of the 1994 Study

Mountain whitefish exposed to pulp mill effluent had higher condition factors, growth (size-at-age), gonad weights, and gonadosomatic index (GSI), compared to fish from the reference site, likely as a result of organic enrichment below the pulp mill. Condition factor, liver weight, hepatosomatic index (LSI), gonad weight, and GSI increased between 1992 and 1994 in fish sampled below the pulp mill. The changes between 1992 and 1994 may indicate increased availability of benthic prey items due to improved pulp

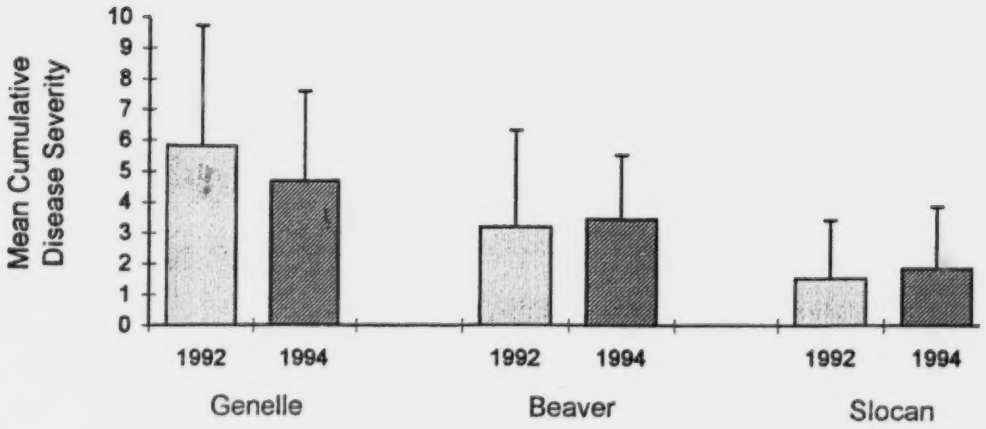

Fig. 1. Mean cumulative disease severity in 1992 and 1994, by reach.

mill effluent quality following completion of secondary treatment and 100% chlorine dioxide substitution in June, 1993.

Mountain whitefish from the Genelle and Beaver Creek reaches of the Columbia River had consistently higher prevalence and severity of abnormalities when compared to the control site on the Slocan River. Mean Cumulative Disease Severity (CDS) was significantly higher at Genelle than at Beaver Creek or the Slocan River reference site, and mean CDS was significantly higher at Beaver Creek than at the reference site ($p<0.001$) (Fig. 1). This pattern of differences held even when the effect of natural aging on CDS was removed from the statistical analysis. The disease survey results were similar to those from 1992, which found a higher incidence of stress-related abnormalities in fish from both Genelle and Beaver Creek, compared to fish from the same reference site on the Slocan River.

CDS did not differ significantly between the 1992 and 1994 studies at any sampling location (Fig. 1). The incidence of some abnormalities, such as inflammatory activity in the liver, melanosis in the liver, granulomas in the liver and kidney, and opportunistic bacterial infections, decreased between 1992 and 1994. The severity of liver and kidney abnormalities also decreased significantly between 1992 and 1994, indicating that the level of chronic irritation experienced by whitefish in the Columbia River has decreased. The prevalence and severity of other abnormalities, however, increased significantly at all reaches between 1992 and 1994 (e.g. haemorrhaging at the base of the fins, severity of external and gill abnormalities, prevalence of gill parasitism combined with significant levels of inflammatory activity).

PCDD, PCDF and PCB concentrations were higher in muscle tissue of fish from the two Columbia River sites compared with the reference site, but all concentrations were low in 1994 (Fig. 2). PCDD and PCDF concentrations declined substantially at the two Columbia River sites compared with 1991 and 1992 data. PCB concentrations have remained stable since 1992. The contribution of PCB compounds to toxicity equivalency units was almost equal to that from PCDD and PCDF congeners in 1994.

Levels of two monooxygenase enzyme activities (EROD and AHH) in the Columbia River mountain whitefish have declined since 1991 and were approaching levels similar to those found at the reference sites by 1994 (Fig. 3). This is indicative of a reduction of inducing materials present in the river over this time period. Although Genelle has statistically higher levels of enzyme activity than Beaver Creek, the levels at Genelle are similar to those at the Slocan River reference site which is a major improvement over 1991.

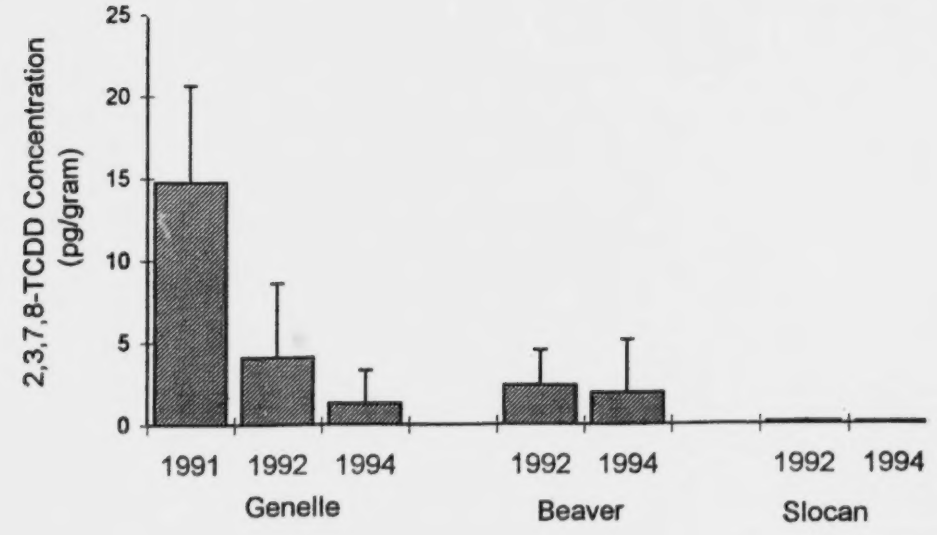

Fig. 2. Time trend of 2,3,7,8-TCDD concentrations in mountain whitefish muscle tissue.

Although organochlorine residues and EROD and AHH activity for the most part correlated at the Genelle site, the size of the enzyme signal in all fish in 1994 was not as high as would be expected if the primary cause of induction was the widely known organochlorine inducers - PCDD, PCDF and coplanar PCBs. The apparent induction seen in the 1994 samples at Genelle is consistent with more transient induction seen around non-bleaching mills and organochlorines are probably a contributing factor rather than the exclusive cause.

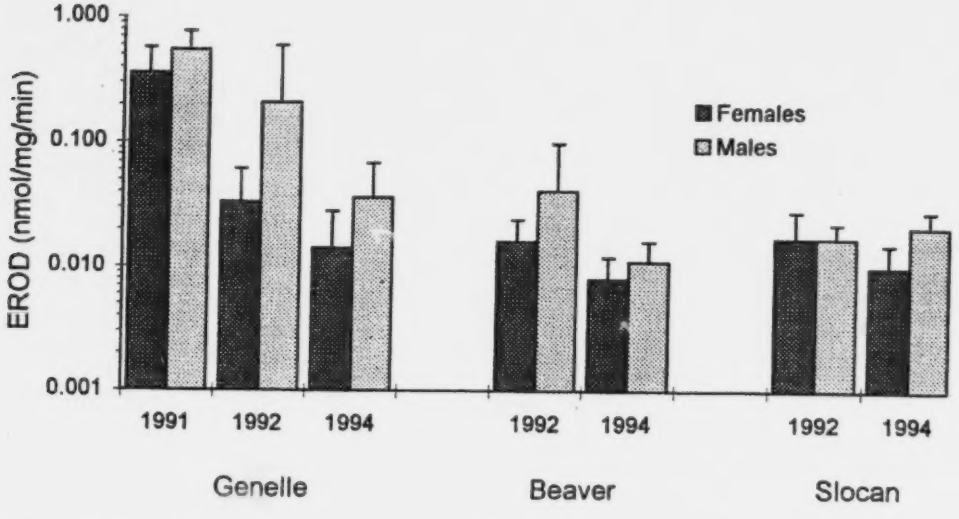

Fig. 3. EROD activity measured in mountain whitefish from the Columbia and Slocan Rivers in 1992 and 1994.

Metal analyses in fish muscle tissue showed that all concentrations were generally highest at the reference site, followed by the Beaver Creek reach below the smelter and then the Genelle reach below the pulp mill. Concentrations of metals (Cd, Cu, Zn, Hg) in liver were typically highest at Beaver Creek, followed by the reference site and Genelle.

Metallothionein correlated strongly with tissue-bound copper and cytosolic cadmium only in fish from the site below the smelter. Comparison of copper and zinc in liver and muscle tissue indicated that copper, and to a lesser extent zinc, was being stored preferentially in livers from fish below the smelter. Smelter slag particles could be a vector for these metals. The 1994 metals results confirmed the 1992 findings, and metallothionein concentration was lower in 1994 compared with 1992 at all sampling locations.

CONCLUSIONS

No evidence of reduced growth, energy storage, or reproduction in fish from the Genelle and Beaver Creek reaches of the Columbia River was found, as the fish from the Columbia River had significantly higher condition factor, growth (size-at-age), liver weight, gonad weight, and GSI than the similarly-aged fish from the reference site. However, the consistently higher mean prevalence and severity of abnormalities in Genelle and Beaver Creek whitefish indicates that Columbia River fish still may experience a decreased quality of environment, as compared to Slocan River fish.

The first two sampling phases of this study document the decline in PCDD and PCDF concentrations and MFO activities in Columbia River mountain whitefish following secondary treatment and 100% chlorine dioxide substitution for elemental chlorine in the bleaching process. Various types of stress-related abnormalities were found in Columbia River mountain whitefish in 1992 and 1994. However, the consistently low levels of abnormalities such as fin erosion, external lesions, and severe liver abnormalities suggest that tissue damage directly due to gas bubble disease, contaminants or mill effluent is not occurring. CDS was not correlated with concentrations of organic contaminants or MFO activities. Relationships between liver and kidney abnormalities and organic contaminants will be looked at separately. Results suggest that CDS measures the cumulative effect of all stresses on the system, including effluent discharges from the pulp mill and smelter, and rapidly changes water levels and high dissolved gas supersaturation from operation of hydro facilities. The final July 1996 sampling phase will confirm the status and trends in chemical contamination and fish health.

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SUITABILITY OF SMALL FISH SPECIES FOR MONITORING THE EFFECTS OF PULP MILL EFFLUENT ON FISH POPULATIONS

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Canadian pulp and paper mills are required to conduct an Environmental Effects Monitoring (EEM) program at effluent receiving waters. An adult fish survey has been included in the program to identify possible changes in whole organism characteristics (growth, reproduction, energy storage, age) of fish exposed to pulp mill effluent. There has been recent concern that larger fish species may not be suitable monitors of local environments because they are very mobile and capable of extensive movement beyond effluent exposure areas. As such, smaller fish species, such as cyprinids and cottids, have been evaluated as possible monitoring species because they exhibit reduced mobility and home range. Recent work has focused on spoonhead sculpin and lake chub of the Athabasca River (AB), as well as trout-perch from the Moose River system (ON). Evaluation of these smaller species was based on abundance, capture efficiency, and responses to mill effluent. Responses were described by comparing whole organism and physiological (MFO induction, sex steroid levels) measurements in fish collected upstream and downstream of the effluent outfalls. Differences in characteristics between exposed fish and reference fish indicated that small species were capable of showing responses to effluent. In addition, graded responses were also observed in fish collected from sites exposed to different concentrations of effluent. However, in the Moose River system where mobility of large fish species is restricted by dams, differences between the responses of trout-perch and larger white sucker were observed. Advantages and limitations of using small fish species for monitoring will be further discussed.

ADULT FISH SURVEY FOR ENVIRONMENTAL EFFECTS MONITORING ON THE ATHABASCA RIVER FOR THE ALBERTA NEWSPRINT COMPANY, WHITECOURT, ALBERTA

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Alberta Newsprint Company (ANC) operates an integrated thermomechanical pulp (TMP), de-inked pulp (DIP) and paper mill near Whitecourt, Alberta, which produces newsprint at a capacity of 620 tonnes/day. Treated effluent and non-contact cooling water are discharged to the Athabasca River at a rate of about 16,000 m^3/day. The study area represents effluent concentrations ranging from 0.01 to 0.2% at low river flow. The Cycle 1 EEM adult fish survey selected lake chub and longnose sucker as the sentinel species. The parameters examined were growth (length, weight and condition), reproduction (gonad size, gonad somatic index, egg size and number of eggs) and energy storage (liver size and liver somatic index) for lake chub in the spring and fall and for longnose sucker in the fall. No evidence of reduced reproductive success was found in either sentinel species. Condition of both male and female lake chub was lower in the study area than the reference area during the fall suggesting decreased somatic (muscle) tissue. The liver somatic index was higher in female longnose suckers in

the study area than the reference area during the fall, which was likely caused by an increased food supply due to organic enrichment in the study area. This response reflects an increase in energy storage suggesting a limited impact.

WILL SENTINEL SPECIES THEORY WORK IN THE ATHABASCA RIVER?

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Federal Environmental Effects Monitoring (EEM) legislation requires all pulp and paper mills to conduct adult fish surveys in which two 'sentinel species' are used as monitors of possible effects from mill effluents or other mill activities. Sentinel species are to be selected from local fish populations which exist in sufficient numbers to allow intensive study within the area of the mill's effluent discharge. Results from adult fish surveys conducted for four pulp mills on the Athabasca River were compared to the requirements set out under EEM for sentinel species. Large bodied fish movement as well as obtaining the minimum sample size of mature individuals was a common problem for all mills. Small bodied fish were also evaluated at two mill study areas where movement was not assessed and effluent exposure was uncertain. Minimum sample sizes were obtained during fall but not in spring when spawning and overwintering would be more likely to show any whole organism response. The Athabasca River is subject to sudden, large variations in flows and water levels. These natural factors influence available habitat for river fish. The use of refugia by river fish has been demonstrated to be common and may exclude them from exposure to effluents. Current effluent quality and dilution, along with other natural factors would make determining fish population effects from Athabasca River pulp mills unlikely.

ENVIRONMENTAL EFFECTS MONITORING ON THE PEACE RIVER FOR DAISHOWA-MARUBENI INTERNATIONAL LTD., PEACE RIVER, ALBERTA

B.M. Shelast[1], M.E. Luoma[1], K.T. Brayford[1] and T. Tarpey[2]. [1]SENTAR Consultants Ltd., Calgary, AB; [2]Daishowa-Marubeni International Ltd., Peace River, AB.

Daishowa-Marubeni International Limited (DMI) operates a bleached kraft pulp mill near Peace River, AB, with a capacity of 1,000 ADt/day. Treated effluent is discharged to the Peace River at a rate of about 60,000 m^3/day. The study area represents effluent concentrations ranging from less than 0.05 to 0.2% at low river flow. The Cycle 1 EEM study included a benthic invertebrate community survey, adult fish survey and effluent toxicity testing. The benthic invertebrate community survey indicated that there was no organic enrichment effects or any other type of effects in the Peace River from the DMI effluent. Some mild organic enrichment was found in a localized area of the river (reference area) likely due to the Town of Peace River sewage effluent. The adult fish survey examined the parameters of growth (length, weight and condition), reproduction (gonad size, gonad somatic index, egg size and number of eggs) and energy storage (liver size and liver somatic index) for the sentinel species of burbot and longnose sucker during the fall. No evidence of reduced reproductive success was found in either sentinel species. Condition of male burbot was lower in the study area than the reference area, but there were no other accompanying changes in other parameters. The liver somatic index was higher in male longnose suckers in the study area than the reference area indicating an increase in energy storage and suggesting a limited impact. The toxicity testing indicated that the effluent concentrations in the Peace River were well below levels that would induce any effect on organisms in the river.

OVERVIEW OF THE DEVELOPMENT OF EXPOSURE PROTOCOLS TO ASSESS THE IMPACT OF PULP MILL EFFLUENT ON FISH

L.H. McCarthy[1], K.R. Munkittrick[2], B.R. Blunt[1], J.J. Jardine[2] and G.J. Van Der Kraak[3]. [1]Department of Fisheries and Oceans, GLLFAS, Burlington, ON; [2]Environment Canada, NWRI, Burlington, ON; [3]University of Guelph, Department of Zoology, Guelph, ON.

In 1992, under the auspices of the Department of Fisheries and Oceans Green Plan Toxic Chemicals Program, a study was initiated, in conjunction with ongoing research, to assess biological effects of pulp mill effluent on fish. Studies in the past had shown that the ability of some pulp mill effluents to disrupt production and circulation of steroid hormones in wild fish may have population-level implications. Once it was determined that existing toxicity bioassays could not predict these responses, new laboratory tests were developed to demonstrate the physiological disruptions found in wild fish. Issues that required research included developing the best laboratory tests capable of predicting effluent impact at the biochemical level, determining the effluent threshold exposure levels for such responses, and assessing whether similar biochemical impacts were occurring with additional types of industrial discharges. Radioimmunoassays were used to measure levels of gonadal and blood hormones in laboratory fish. Interlaboratory collaboration has resulted in significant refinement to the protocol and has increased the transferability of the assay. Optimization trials are ongoing and include experiments addressing test vessel size, bioassay duration, static versus replacement exposures, species feasibility, feeding status, etc. Effluent was collected from a series of Canadian mills with differing process and waste treatment strategies and the current study is presently trying to establish a relationship between anomalous hormone levels observed in the test fish and specific mill processes. Strengths and limitations of the exposure protocol will be examined with subsequent discussions of future research direction.

MONITORING PULP AND PAPER MILL EFFLUENT QUALITY BY LABORATORY BIOTESTS. I. WHOLE ORGANISM RESPONSES

P.H. Martel, T.G. Kovacs, S.J. Gibbons, B.I. O'Connor and R.H. Voss. Pulp and Paper Research Institute of Canada, Pointe-Claire, QC.

This paper reviews the results of investigations evaluating the relationship between mill manufacturing technology and effluent quality as determined by a series of short-term and long-term laboratory tests. Short-term EEM sublethal tests were used to test the effluent from mills representing the major types of pulping/bleaching processes and effluent treatment options in Canada. Fathead minnow life cycle experiments were used to compare the effluent from a thermomechanical pulp (TMP) mill as well as effluent from a bleached kraft mill before and after process and treatment changes. The ability of short-term tests to predict responses was also examined. The results indicated that recent manufacturing and treatment changes made by Canadian mills have resulted in the discharge of effluents which are approaching a margin of safety compatible with the aquatic environment.

MONITORING PULP AND PAPER MILL EFFLUENT QUALITY BY LABORATORY BIOTESTS. II. HEPATIC MFO RESPONSES IN FISH

P.H. Martel, T.G. Kovacs, B.I. O'Connor and R.H. Voss. Pulp and Paper Research Institute of Canada, Pointe-Claire, QC.

This paper reviews the results of investigations evaluating the relationship between mill manufacturing technology and the potential of effluents discharged by mills to cause MFO induction in fish. A

laboratory-based exposure procedure was used to quantify the relative MFO induction potencies of effluents from pulp and paper mills using different types of pulping, bleaching and treatment options. The effects of a variety of kraft bleaching operations as well as secondary treatment on the potential of final mill effluents to cause increased MFO activity were given special consideration. The results indicated that effluents currently discharged by mills caused only marginal MFO induction. No clear trends with respect to manufacturing technology were evident. Secondary treatment was found to substantially reduce the potency of the effluents to cause increased hepatic MFO activity. Detailed investigations at selected mills provided insights into the source and identity of MFO inducers.

USING CAGED BIVALVES FOR ENVIRONMENTAL EFFECTS MONITORING AT PULP AND PAPER MILLS: RATIONALE AND HISTORICAL PERSPECTIVE

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ABSTRACT

There is general agreement between regulatory agencies and industry that new, and integrated approaches are necessary to monitor and assess environmental conditions in the vicinity of pulp and paper mill effluents. Traditional monitoring approaches have considerable uncertainty, and any single method is clearly not adequate. Current monitoring approaches utilize laboratory bioassays, benthic community structure and adult fish to surveys to estimate exposure and effects. There are several problems even with this integrated approach. Sources of uncertainty include extrapolation from laboratory bioassays to site-specific conditions, availability of appropriate fish species, and difficulties in interpreting effects and exposure data from benthic communities. Perhaps most frustrating is the use of fish to characterize chemical bioavailability. Numerous fish samples have been analyzed and detectable concentrations of the chemicals of concern have only been found in a small percentage of the samples. Often, fish are not even found in the areas of concern. Measurements of biochemical indicators have been inconsistent, and there is no clear relationship between the induction of these biomarkers and fish population effects. Adult fish surveys have not been able to answer the questions most commonly asked by government, industry and the public 'Are conditions getting better, worse, or staying the same?' This paper will provide the rationale, historical perspective, and an example of using caged bivalves as a new approach to reduce uncertainty in monitoring and assessing pulp and paper mill effluents. The historical use of caged bivalves in aquatic monitoring programs will be reviewed. In addition, results will be presented from a recent caged mussel study conducted in the Pacific Northwest to evaluate conditions in the vicinity of effluents discharged from a pulp and paper mill.

INTRODUCTION

While the stated object of the Environmental Effects Monitoring (EEM) Program in Canada is to test the effectiveness of current regulations on fish health and fish habitats, measuring fish may not be the most appropriate method for making that assessment. As contradictory as that might seem, measuring water concentrations of chemicals is not the best way to assess water quality. Biological indicators were originally developed as a measurement tool for chemicals in water and sediment when the concentrations were below detection limits of currently available analytical techniques (Phillips, 1980). Biological indicators integrate and concentrate bioavailable chemicals and add a biological component to the chemical analyses. Field bioassays with caged bivalves maximize the utility of a bioindicator approach and minimize the uncertainty associated with traditional laboratory and field methods. Caging facilitates synoptic measurements of exposure and effects over space and time. This approach combines the experimental control of laboratory bioassays and the environmental realism of field monitoring. Over the past 20 years the design and methodology has been refined to include mussels,

clams, and oysters; marine, estuarine, and freshwater habitats; and depths to 80 m. Transplanting caged bivalves in the immediate vicinity of outfalls will guarantee an exposure and almost certainly produce measurable tissue burdens if the chemicals are biologically available. Placing test animals along chemical gradients will also help identify potential sources. Field studies with caged bivalves facilitate measuring bioaccumulation and bioeffects because [1] bivalves are easy to collect, cage, and measure, [2] bivalves will accumulate bioavailable chemicals, and [3] bivalves can be measured for adverse effects (Salazar and Salazar, 1995). Recently, we have described the application of caged bivalves to support ecological risk assessments (Salazar and Salazar, in review) and to characterize exposure and effects associated with pulp and paper mill effluents (Salazar and Salazar, in press).

It is timely to consider the use of new and innovative approaches for monitoring effluents because the results of Cycle I monitoring are currently being reviewed. Results of the review will be used to improve the approach for Cycle II of the EEM Program. Further, it is always helpful to return to the questions that are being asked as part of any monitoring program to ensure the best methods are being used. Quantifiable, scientifically defensible, and easily understood answers are needed that reduce the uncertainty in the risk assessment process. The questions should also be asked within the context of an ecological risk assessment: [1] What is the characterization of exposure in the major environmental compartments — water, sediment, and tissue? [2] What is the characterization of effects in ecologically significant receptors? [3] Are conditions getting better, worse, or staying the same?

Government, industry, and the public are frustrated with the high degree of uncertainty in traditional approaches. Many expensive fish surveys have been conducted only to result in non-detectable concentrations of chemicals in the tissues or biomarker measurements of chemical exposure that are ambiguous, difficult to interpret, and of limited value. Some tagged fish studies have been conducted where none of the tagged fish were recovered. Cost per unit effort is very high. Mixing zones are often poorly defined and chemical analysis of discrete water samples do not address the integrated exposure and effects associated with chemicals of concern. Every extrapolation adds to the uncertainty. Numerous extrapolations must be made on data from laboratory bioassays, analyses of benthic community structure and from monitoring various ecological receptors in natural fish populations. These types of monitoring tools characterize chemical exposure and associated effects with relatively high levels of uncertainty.

RATIONALE

One way to reduce the uncertainty associated with traditional monitoring methods is the use of field bioassays with caged bivalves. Field bioassays bridge the gap between traditional laboratory bioassays and field studies, they combine the experimental control from laboratory bioassays and the environmental realism from field monitoring into a cost-effective monitoring tool (Fig. 1). The main advantages of field bioassays with caged bivalves are [1] the ability to obtain site-specific measurements and establish site-specific links under environmentally realistic conditions; [2] strategic placement of test animals along suspected chemical gradients where they do not move, [3] testable hypotheses, and [4] test species are always available and the same species can be used in many different environments. In the field bioassay approach, exposure is characterized by measuring chemicals in tissues, and effects are characterized by measuring sublethal endpoints like growth. There is a greater likelihood of accurately evaluating the extent of bioavailable chemicals because bivalves, such as mussels, efficiently concentrate and integrate chemicals. Strategic placement of caged bivalves integrates exposure and effects over space and time (Fig. 2). There is relatively no additional expense associated with extending exposure times, so field bioassays can be conducted until chemical equilibrium has been reached. Most laboratory bioassays have been designed to minimize cost and exposure time. However, it is uncertain whether chemical equilibrium has been reached in test animals and whether the measured response reflects realistic exposure conditions.

Bioaccumulation provides the most direct estimate of biologically available chemicals. It provides a

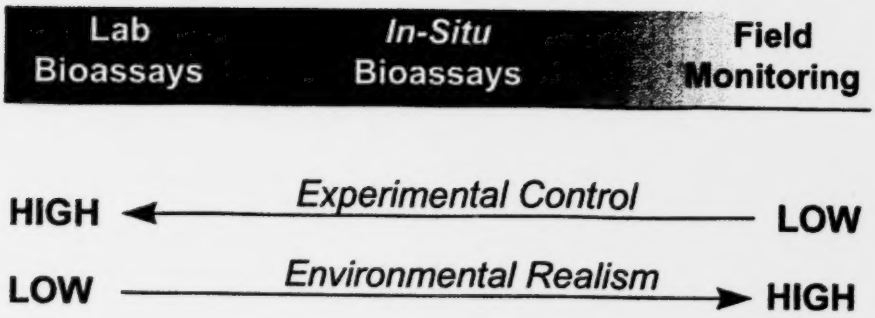


Fig. 1. Bridging the gap between traditional laboratory bioassays and field monitoring with *in situ* field bioassays.

direct link between the external environment (i.e., water and sediment) and the receptor sites (i.e, the internal environment of the organism). Bivalves integrate chemical exposure over time as they filter the water for food and sequester the chemicals along with the food. Both natural and transplanted populations of bivalves have been used for bioaccumulation studies around the world (Phillips, 1980). Growth represents an integration of all internal biological processes and can be associated with water, sediment, and tissue concentrations of chemicals.

There is a perception that because bivalves are routinely used in bioaccumulation studies, they are insensitive to chemicals. A number of recent studies have shown that bivalves are equally or more sensitive than other commonly used bioassay organisms, and growth is an integrated effects endpoint that can be readily quantified. For example, Burgess and Morrison (1994) have shown that for marine sediments, although clam (*Mulinia lateralis*) and amphipod (*Ampelisca abdita*) acute endpoints are relatively similar, the sensitivity is increased in clams when growth is used as a sublethal endpoint. In their tests on six freshwater pulp mill effluents, McKinney and Wade (1996) showed that 9 d mussel mortality (*Anodonta imbecilis*) demonstrated greater sensitivity than 7 d daphnia mortality (*Ceriodaphnia dubia*). On a tissue residue basis, Salazar and Salazar (in review) have shown that an 84 d growth endpoint in caged mussels (*Mytilus galloprovincialis*) was an order of magnitude more sensitive to tributyltin (TBT) than the mortality endpoint in four marine amphipod species reported by Meador et al. (1996).

Bivalves are sedentary and have hard shells. This makes them easy to collect, cage, and measure. Using farms or culture facilities as the source of test animals has the added benefit of a population with a known genetic and environmental history. There is also an extremely large database for exposure and effects data from both laboratory and field studies. Caging does not affect chemical exposure to bivalves as it does with fish, caged fish do not have the freedom to feed in their usual way because of confinement. Further, most bivalves have a limited capacity to metabolize petroleum hydrocarbons (PAHs) and other chemicals in mill effluents that have been associated with MFO induction in fish. The main problem in using biomarkers of chemical exposure like MFO is that they are too ephemeral and persist for only a short period of time after exposure. Recent work has also suggested that there is no direct link between MFO induction and population effects (Munkittrick et al., submitted).

It is important that the chemicals in the effluent plume be accurately mapped. Likewise, it is necessary to accurately evaluate the effects from that chemical exposure. Caged mussels provide a direct method of mapping the extent of chemical contamination, defining mixing zones, and assessing the subsequent biological effects. Caged bivalves accumulate and respond to only biologically available chemicals.

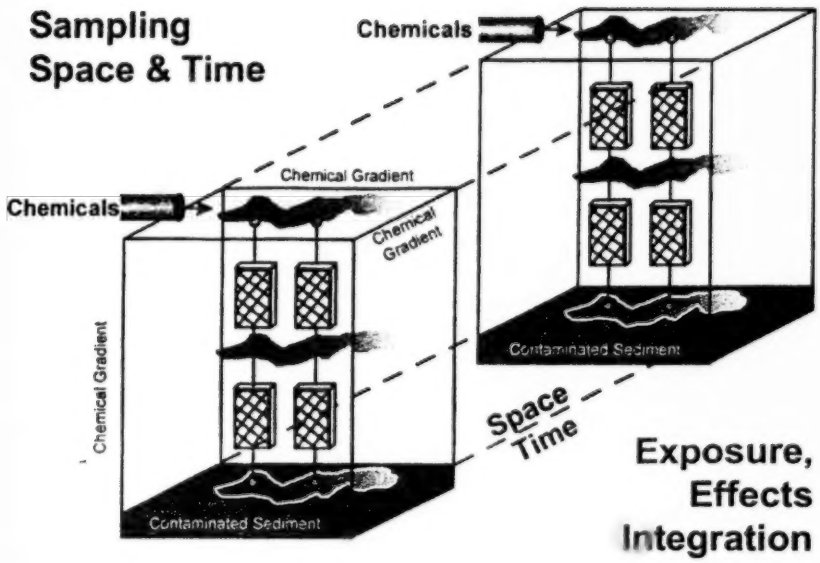


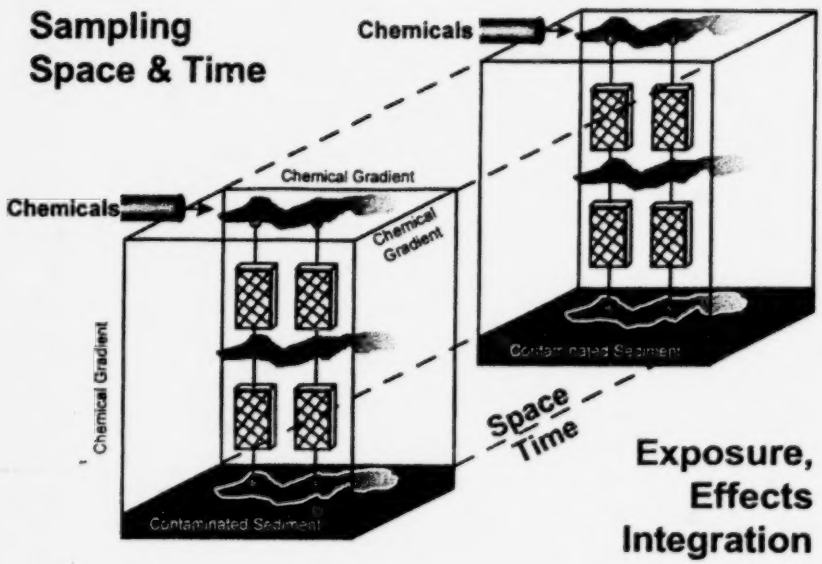
Fig. 2. Using caged bivalves to monitor chemical exposure and biological effects over space and time through integrated measurements.

Routine analysis of thousands of water samples cannot reveal which chemicals are bioavailable or the effects of exposure to multiple chemicals. It is very difficult to define the extent of chemical contamination or map the effluent plume using natural fish populations because they are so mobile. This mobility results in high uncertainty associated with the exposure period and position.

Historical Perspective

Since the initiation of environmental monitoring programs for pulp and paper mills, fish have been the emphasis for monitoring receiving waters. Techniques for collecting and analyzing tissues of resident fish populations are well established and used routinely. However, it is becoming increasingly apparent that using mobile species may not be the best approach. Scandinavian scientists were among the first to associate effects on growth and reproduction in fish with chemicals in effluents. They also associated specific biomarkers with those organismal effects. This approach was adopted by Canadian regulatory agencies early in the EEM Program. Unfortunately, even though significant progress has been made in quantifying the endpoints, and even the compounds responsible for these effects and MFO induction, there is still no clear relationship between induction and population effects (Hodson, 1996; Munkittrick, submitted). This could have been predicted several years ago on the basis of the mobility of the fish populations and the short duration of MFO induction after exposure ceases. The Scandinavians have shown additional foresight by developing an integrated monitoring program that includes bivalves and fish (Rantio et al, 1996). Integrated programs at several levels of organization have been suggested previously (Chapman, 1996; Hall, 1996).

Historically, caged bivalves have been used to monitor pulp and paper mills in freshwater and marine environments, but the emphasis has been on bioaccumulation of chemicals in tissues. The largest data set for bioavailable chemicals comes from Finland, where they have been using this approach since 1984 (Herve et al., 1988; Herve, 1991; Herve et al., 1996). They primarily use the freshwater mussel *Anodonta piscinalis* and the method referred to as "mussel incubation." Much work has been done in



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Canada with the freshwater mussel *Elliptio complanata,* but again much of the emphasis has been on bioaccumulation (Metcalfe and Hayton, 1989). Another complicating factor is that much of the information is either unpublished or found in the grey literature. Other examples of using freshwater bivalves to monitor similar effluents include the use of *Corbicula fluminea* to evaluate effluents from a wood treatment facility discharged to the San Joaquin River, CA (Hayward et al., 1996), *Anodonta cygnea* to evaluate extractable organic halogens discharged into the Ton River in France (Hayer and Pihan, 1996) and *Hydridella menziesi* to assess accumulation and depuration of resin acids in a freshwater pond in New Zealand (Burggraaf et al., 1996).

The Proceedings of the 17th Aquatic Toxicity Workshop (Parker et al., 1991) contained a draft proposal for the Canadian EEM Program that included field bioassays with caged bivalves to estimate chemical exposure. This approach was excluded from the final EEM regulations, the reasons for exclusion were unclear. Field bioassays with caged bivalves are currently required by several countries, but usually to document exposure by measuring chemicals in their tissues. This approach has also been required at one U.S. pulp and paper mill as part of their NPDES monitoring program. Caged mussel studies were included in the permit because no natural populations could be found to demonstrate whether chemicals were biologically available and having adverse effects. In their effort to understand the fate of chemicals once the effluent is discharged, the Canadian EEM Program requires both the effluent and receiving waters to be monitored. Except for the one particular case mentioned above, the major effort in the U.S. is directed toward characterizing only the effluent. Field bioassays with caged bivalves could enhance monitoring programs in both Canada and the U.S. In Canada, this shift in focus would reduce the uncertainty by decreasing the reliance on fish to characterize exposure and effects in the receiving waters. In the U.S., this shift in focus would reduce the uncertainty by decry bioassays to characterize the receiving waters.

Example

In the U.S., one pulp and paper mill has been required to use field bioassays with caged mussels. Mussels (*Mytilus trossulus*) were collected from an uncontaminated reference site, measured, placed in cages, and transplanted to seven sites. These included two reference sites and five sites in the vicinity of the effluent discharge at distances of 100, 300, 600, 800, and 1000 meters from the outfall. A 60 d exposure period was used. Three replicates of 100 animals each were deployed at each site. The following mussel metrics were used to determine the effect of chemical exposure on growth: whole-animal wet-weight, shell length, shell weight, and tissue weight. All mussels were measured at both the beginning and the end of the test. Measurements included whole-animal wet-weights and shell lengths and were recorded and tracked on an individual basis. An additional 300 mussels were measured at the beginning of the test for whole-animal wet-weight, length, tissue weight, and shell weight. The tissue weights from these animals were used to estimate tissue weights of the caged mussels at the beginning of the test. Tissues from these 300 mussels were analyzed for the chemicals of concern to determine background concentrations at the reference site. Individual tissue and shell weight measurements were also made at the end of the test for all deployed mussels. After the 60 d deployment, mussel tissues were chemically analyzed to determine concentration of selected chemicals, including dioxins (PCDDs) and furans (PCDFs). This facilitated estimates of bioavailability and comparisons between beginning and end-of-test tissue burdens.

Results can be summarized as follows [1] compared to the initial tissue concentrations, total PCDD/PCDF concentrations after 60 d of exposure were significantly elevated in mussels within 1000 meters of the effluent discharge, [2] in all mussel tissues, a large percentage of total PCDD/PCDF was octachlorodibenzo-p-dioxin (OCDD), [3] based on the Toxic Equivalence Concentration (TEQ), the concentrations of dioxin and furan compounds in mussel tissues were about an order of magnitude below the predicted low risk TEQ of 50 ng/kg, and [4] based on changes in whole-animal wet-weight and shell length, mussels at the test sites had significantly lower growth than the reference sites.

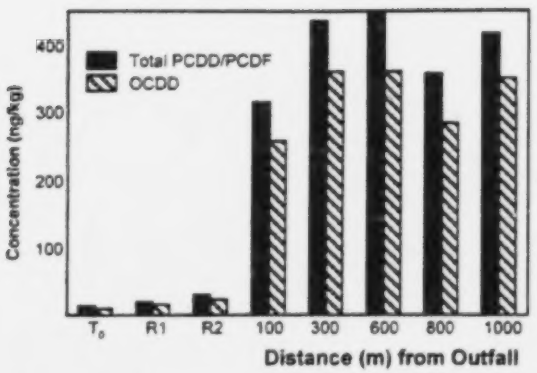

Figure 3. Concentrations of Total PCDD/PCDF versus OCDD in mussel tissues at the start (T_0) of the test, deployed at two reference sites, and at various distances from the mill outfall.

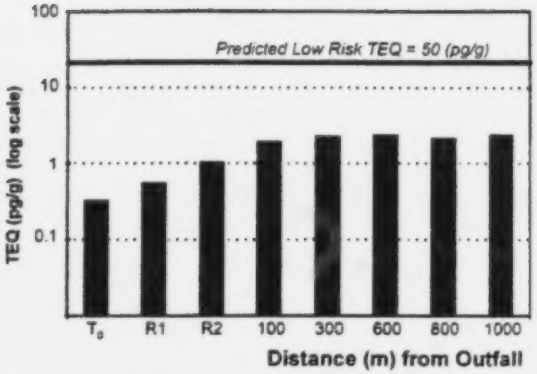

Figure 4. Toxic equivalence concentration (TEQ) for dioxins and furans in mussel tissues compared to the low risk TEQ of 50 pg/g.

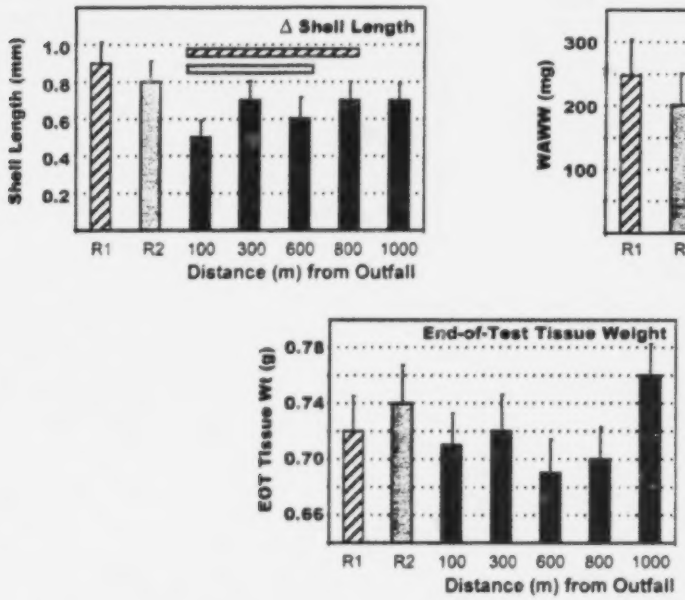

Figure 5. Differences in mussel growth at two reference sites and at various distances from the mill outfall using three different metrics: change in shell length, change in whole-animal weight, and end-of-test tissue weight. Horizontal bars indicate statistically different from the reference sites.

Statistically different from R1 Statistically different from R2

The concentrations of PCDD/PCDFs in mussel tissues increased by about an order of magnitude over the 60 day exposure period (Fig. 3). The maximum concentration measured in mussel tissues was about 400 ng/kg. Interestingly, the highest concentrations were not found at the site closest to the effluent pipe. OCDD constituted approximately 80 percent of the total PCDD/PCDF at each test site. Although mussels at the mill sites showed statistically significant elevations in dioxins/furans, it does not appear that these tissue burdens are environmentally significant. The predicted low risk Toxic Equivalency Concentration (TEQ) is 50 ng/kg (U.S. EPA, 1993). The TEQs determined for mussels in the vicinity of the mill were about an order of magnitude below this predicted concentration (Fig. 4).

Although the lower growth rates measured in the vicinity of the mill effluent were statistically significant, these differences may not have been environmentally significant because the concentration of PCDD/PCDFs in tissues were not environmentally significant and the absolute differences in mussel metrics were very small. In this study, changes in whole-animal wet-weight were the most discriminating metric and end-of-test tissue weights were the least discriminating (Fig. 5). Based on our most recent work in other areas with slow-growing mussels, we expected tissue weights to be the most discriminating. Two factors may have precluded the ability of this metric to be more discriminating. First, the test was conducted from January through March when mussels approached their primary spawning season. Early spawners could have added to the variability in end-of-test tissue weights, and thus prevented a more discriminating statistical analysis. Second, the overall size range was about 8 mm and this could have added to the variability in tissue weights. To reduce these potential confounding effects, fall deployments with a size range of 5 mm or less has been recommended (Salazar and Salazar, 1995).

SUMMARY AND CONCLUSIONS

We have shown the utility of caged bivalve monitoring for pulp and paper mill effluents by providing the rationale, historical perspective, and an example. The results of the example provided here demonstrate the discriminating power and the significance of initiating these tests with mussels of uniform size and health. In addition, it is important to consider the time of year and reproductive status of test animals during the interpretation of test results. Since tissue burdens provide a direct estimate of bioavailable chemicals in water and sediment they are being used more often in environmental risk assessments. Some regulations are also shifting toward a tissue residue basis. Field bioassays with caged bivalves are a potentially powerful tool in the risk assessment process, but data interpretation depends on understanding the physical and biological processes that affect chemical exposure and associated biological effects.

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AN INVESTIGATION INTO THE GENETIC TOXICITY OF PULP MILL EFFLUENT ON JUVENILE CHINOOK SALMON (*Onchorhynchus tshawytscha*) USING FLOW CYTOMETERY

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A mobile laboratory was utilized to conduct on-site bioassays at the furthest upstream pulp mill on the Fraser River in British Columbia. Uncontaminated river water was used to dilute treated effluent as discharged from the final diffuser pond. The range of concentrations used spanned those that could be encountered by juvenile chinook salmon (*Onchorhynchus tshawytscha*) which overwinter in the upper Fraser River mainstem. A single cohort of juvenile (8-10 g) chinook salmon from the Penny Hatchery were divided into two groups. The hypoxic group received 65% oxygen saturated water, while the normoxic group received ambient 88% oxygen saturated water. Both groups were exposed over a period of 30 d to effluent concentrations of 2, 4, 8, and 16%, while the controls received uncontaminated river water. At the end of the bioassay, all fish were sacrificed. Blood was then collected and couriered fresh to the West Vancouver Laboratory for analysis by flow cytometry. The DNA was treated with a proprietory enzyme and stained with propidium iodide. Both the normoxic and hypoxic groups showed significant dose-dependent clastogenic damage. The hypoxic and normoxic groups did not differ in their response to the various concentrations of BKME. A concentration response curve of the form

$$Y = -0.25828 + 0.06050X - 0.00225X^2$$

was determined from the hypoxic group data set. Genetic implications of potential mutagenic damage caused by BKME in natural populations of chinook salmon in the upper Fraser River system is explored. The flow cytometer is discussed as a useful tool in the detection of genotoxic damage both in lab and field studies.

ENVIRONMENTAL EFFECTS MONITORING: TRENDS IN AN ECOSYSTEM'S RESPONSE WITH TECHNOLOGICAL CHANGES AT A PULP MILL

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Studies investigating environmental effects have been undertaken since 1990 at the Grande Prairie, AB, kraft pulp mill. This paper presents both the results of the most recent studies conducted in the Wapiti/Smoky River system (1995) and the changes observed over the past six years in the biological communities in these receiving waters. Three main components or sets of variables have been collected over the course of these studies includes [1] chemical measurements in the effluent, in the receiving and reference waters and in fish tissues, [2] biological surveys of organisms living in these waterbodies, and [3] sublethal toxicity testing of the mill's treated effluent. The relationships and trends among these are explored.

Immediately after the first year of studies in 1990, technological changes were adopted by the mill in order to improve the quality of the final effluent, and technological upgrades have since been ongoing. The improvements at the mill have been reflected in both the effluent quality and the quality of the receiving waters. Levels of such contaminants as dioxin and furan congeners, chlorinated phenols, and resin and fatty acids have continuously declined in both the effluent and the receiving waters. Concentrations of these compounds have also been declining in fish tissues. Though improvement to the fish community (increased diversity) may be due to the immigration of fish back into the receiving waters after the 1:100-year flood event in 1990 had flushed fish from the system, a general improvement

at the population level (growth, survival and reproduction) has also been observed. Results of toxicological testing have reflected the improving quality of the effluent. The benthic community continues to demonstrate a response to mild nutrient enrichment.

BENTHIC INVERTEBRATE COMMUNITY SURVEY FOR ENVIRONMENTAL EFFECTS MONITORING ON THE ATHABASCA RIVER FOR ALBERTA NEWSPRINT COMPANY, WHITECOURT, ALBERTA

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Alberta Newsprint Company (ANC) operates an integrated thermomechanical pulp (TMP), de-inked pulp (DIP) and paper mill near Whitecourt, AB, which produces newsprint at a capacity of 620 tonnes/day. Treated effluent and non-contact cooling water are discharged to the Athabasca River at a rate of about 16,000 m^3/day. The study area represents effluent concentrations ranging from 0.01 to 0.2% at low river flow. The Cycle 1 EEM benthic invertebrate sampling design was based on the annual historical monitoring design which was established in 1989, prior to mill startup. During the fall of 1994, there appeared to be an effect of mild organic enrichment in the study area from the ANC effluent. Tolerant taxa, mainly Chironomidae, as well as the intolerant taxa of Ephemeroptera and Trichoptera, increased in numbers, chlorophyll *a* increased, and the number of taxa increased or remained the same at study area sites in comparison to reference areas sites, in response to the mild organic enrichment. These increases also caused shifts in the feeding group structure between sites. Low summer flows during 1993 resulted in a similar natural increase in organic enrichment in the river. This observation emphasizes the significance of physical factors related to river flow throughout the year when determining the extent of the productivity of the river, whether in the reference area or the study area.

TIERED SPATIAL DESIGN AND MULTIPLE COMPONENT ASSESSMENTS TO DISTINGUISH TWO CLOSELY SITUATED PULPMILL DISCHARGES

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Wastewaters, receiving waters, sediments, benthic invertebrate and populations, were examined as part of Cycle 1 of the federal EEM initiative conducted on behalf of two pulpmills discharging within close proximity to a northern reservoir. Challenges in distinguishing potential individual effects were complicated by proximity of discharges, complexity of ecological region of discharge and overall reservoir system, temporal and spatial confounding influences, etc. Program design and data interpretation were addressed through use of a tiered spatial gradient design. Comparison of similar reference and control regions as part of the tiered spatial design was important in elucidating community structure and receiving environment trends. A comprehensive examination of the benthic community structure involved the combined assessment of several biological endpoints (total numbers, relative proportion or taxonomic groups, species richness, and indicator species) in conjunction with physical and chemical sediment information. The tiered spatial approach, based on a reservoir ecological model (Thornton et al., 1981), involved three combined spatial perspectives and assessed community structure differences and separate reservoir and mill-related phenomena. The individual influences of the two mills were ascertained based on subtle but distinctly different signatures of their benthic community structure and sediment quality compared to ambient reservoir profiles.

SENSORY EVALUATION OF FISH EXPOSED TO PULP AND PAPER MILL EFFLUENT: A CASE STUDY OF METHODS USED FOR ENVIRONMENTAL EFFECTS MONITORING

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The federal Environmental Effects Monitoring (EEM) program, as a component of the Fisheries Act, Pulp and Paper Effluent Regulations, requires sensory evaluation of tainting at Canadian pulp and paper mills where there have been historical complaints of fish tainting or altered fishing efforts. Sensory evaluation tests were conducted in British Columbia (3), Alberta (2), and Nova Scotia (1) as a requirement of the first EEM cycle. Each test component yielded considerable variability. Nevertheless, there was a high degree of similarity of the sensory evaluation results at all the mills after substantial process changes and improved effluent treatment. Significant tainting of two fish species occurred within 3 h and at exposure concentrations less than 0.08% (v/v) effluent at one location. Tertiary treated effluent discharged from one mill continued to create a significant taint in resident fish. Adult and juvenile fish appear to be attracted to and reside in effluent plumes immediately downstream of the outfall at some mills. Sensory evaluations may be the most sensitive monitoring tool available to integrate effluent exposure and assess the usability of the valuable sports, recreation, commercial and native food and ceremonial fisheries resources. Standard sensory evaluation test methods and fish exposure conditions to pulp and paper mill effluent are required to improve test sensitivity, confidence, power and reduce variability. A proposal to focus on EEM cycle 2 programs to evaluate the use of the fisheries resources is presented.

DIVERGENT APPROACHES TO EEM INVESTIGATIONS: HOLISTIC SCIENCE AND ECOLOGICAL RELEVANCE

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A discussion of the merits of applying holistic and ecologically relevant biological approaches and practices to EEM investigations. Examples of divergent approaches, including a variety of biological and statistical tools and approaches used as design and decision criteria are discussed. Various ecological and statistical models, biologically and statistically-driven criteria for decisions, alternatives to random, paired-block sampling, spatial and gradient analyses, pooling of data points and samples vs individual analysis, and data reduction for better model-fit vs data integrity with compromised model-fit are discussed. The ramifications of these divergent approaches on decision criteria, assessment of ecological relevance, cost effectiveness and further EEM investigations are discussed and evaluated.

PULP AND PAPER EFFLUENT TRACERS IN FISH

Session co-chairs: I. Cloutier and S. Humphery

ORGANIC TRACE COMPOUNDS IN FISH AND INCUBATED MUSSELS FROM PULP MILL RECIPIENT WATERS

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Bioaccumulating organochlorine compounds (OCC) from bleaching were studied in fish and incubated

mussels from recipient watercourses of pulp mills in Finland during 1978-1995. For comparison, mill effluents, sludges and recipient sediments were also analyzed. The measured OCC concentrations decreased greatly due to process improvements during the time. At present, the modern mills use most commonly ECF bleaching and secondary treatment. They still discharge some amounts of OCCs.

Among chlorophenolics, chloroguaiacols in fish and mussels were best indicators of the exposure to bleaching mill effluent. Three most commonly occurring chlorophenols, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol were nonspecific to pulp mills, while combustion and especially wood preservation caused their residues, as well. Biomethylation products of chlorophenolics, chloroanisoles (PCA) and chloroveratroles (PCV) were main causes of fish tainting in pulp mill exposed watercourse. However, PCVs in fish were more characteristic to pulp mill exposure alone. Among dioxins and furans from pulp mill, two nontoxic tetra-CDDs and the toxic 2,3,7,8-tetraCDF were major congeners. Contents of toxic PCDDs and PCDFs in Finnish recipient samples were very low and originated mainly from combustion and wood preservative chlorophenols. Notable concentrations of polychlorodiphenyl ethers (PCDEs) in fish and mussels in one watercourse were not caused from pulp mill but from chemical manufacture of tetrachlorophenol for wood preservation.

Major chlorohydrocarbons from pulp bleaching were chlorinated cymenes and cymenenes, which were abundant in recipient biota and sediments. Other specific pulp mill waste products were chlorinated alkyl bibenzyls, alkylnaphthalenes and alkylphenanthrenes (e.g. chlororetenes) which were found in recipient biota. Also chlorinated alkylfluorenes, alkyldibenzofurans and alkyldibenzothiophenes were found as pulp mill originated trace substances. Toxaphene congeners (PCCs) were proven not to originate from pulp mill.

APPLICATION OF RESIN ACIDS AS INDICATORS OF ENVIRONMETNAL EXPOSURE TO PULP AND PAPER WASTE DISCHARGES

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The introduction of elemental chlorine free (ECF) bleaching has substantially decreased the concentrations of organochlorine compounds present in bleached kraft pulp and paper mill wastewaters and therefore made these constitutents more difficult to utilize as tracer compounds in recipient biota. Resin acids remain detectable in treated effluents from both non-bleached and ECF softwood pulp and paper mill wastewaters. These compounds readily bioaccumulated in exposed fish. Bile is the major bioconcentration matrix, magnifying water-borne resin acid concentrations by approximately 500,000 times. Bioconcentration factors for resin acids in other fish tissues are several order of magnitudes lower.

Tissue bioconcentration behaviour was found to be comparable in four species (trout, eel, goldfish and mussel) and was well-correlated between laboratory-scale and field studies. The concentrations and composition of these compounds in bile exhibited a strong linear relationship with resin acids in receiving waters and in tissues of exposed fish. These relationships were utilized to successfully predict concentrations of resin acids in the water and fish tissues from another recipient. Results from our studies therefore indicate that resin acids may be suitable tracer compounds to determine recipient biota exposure to softwood pulp and paper wastewaters.

RESULTS FROM TRACER STUDIES IN EXPOSED AND REFERENCE FISH AT MANY CANADIAN PULP AND PAPER MILL SITES

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In the first cycle of a regulated monitoring program for Canadian pulp and paper mills, a chemical tracer was required to be measured at a all sites where fish could move freely between reference and effluent exposure sites. Tracer analyses were conducted on tissue from 27 species of organisms (finfish or shellfish) at 67 mills with varied pulping/papermaking processes and different types of receiving environments, riverine, lacustrine, estuarine and marine. Most mills measured resin or fatty acid concentrations in fish liver or bile while some analyzed for chlorophenols, chloroguaiacols or dioxin and furan congeners. Principal component analysis was applied to the multivariate data. The results indicate that while chemical tracers can be used to detect exposure at some sites, habitat factors and other variables related to mill process and effluent treatment reduce their effective application at other sites. Lessons learned from the detailed review of first cycle fish tracer data will be discussed, and recommendations for future monitoring cycles will be presented.

PULP AND PAPER MILL EFFLUENT TRACERS IN FISH: AN OVERVIEW OF EFFORTS TO EXAMINE ISSUES AND RECOMMEND SOLUTIONS IN A REGULATED ENVIRONMENTAL EFFECTS MONITORING PROGRAM IN CANADA

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During the first cycle of the Canadian regulated Environmental Effects Monitoring Program, pulp and paper mills experienced difficulty with meeting a regulated requirement to analyze for effluent tracers in fish. The purpose of analyzing a tracer in fish tissue is to verify that fish collected in effluent receiving areas have been exposed to effluent and fish collected in the reference area have not. A fish tracer was required at all mill sites where fish could move freely between sampling locations. This poster describes some of the problems encountered with the fish tracer requirement and gives an overview of the efforts of a small working group established to examine the issues and develop recommendations for future cycles of the regulated Environmental Effects Monitoring Program in Canada.

PROBLEMS ENCOUNTERED VERIFYING EXPOSURE IN FISH TO EFFLUENT THROUGH THE USE OF CHEMICAL TRACERS AT BLEACHED-KRAFT PULP MILLS EMPLOYING SECONDARY TREATMENT TECHNOLOGIES

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The federal Environmental Effects Monitoring (EEM) program, a component of the Fisheries Act Pulp and Paper Effluent Regulations, requires mills to verify through the use of a tracer that the fish samples collected have been exposed to effluent in the near- and far-field areas, and to verify the lack of exposure at reference areas. Three bleached kraft pulp mills, two in Alberta and one in Saskatchewan, attempted to verify fish exposure to effluent through the use of chemical tracers. Resin and fatty acids

(RFAs) were selected as x tracers at all three pulp mills, the studies in Alberta measured RFAs in fish bile, and in Saskatchewan RFAs were measured in fish flesh. In the Alberta studies, concentrations of RFAs detected in bile ranged from <0.05 to 0.70 mg/kg in mountain whitefish and <0.05 to 0.45 mg/kg in longnose sucker, considerably less than concentrations measured in other fisheries studies of rivers receiving secondary treated pulp mill effluent. A lack of detectable levels of most RFAs in effluent, together with the low concentrations of RFAs in bile, indicate that RFAs need to be re-assessed as suitable chemicals for tracers at the Alberta sites. In Saskatchewan, RFAs were not detected in fish flesh. Other indicators of exposure analyzed for the studies included chlorophenols in fish flesh and bile, and the induction of a mixed function oxidase enzyme (EROD). Concentrations of chlorophenols in both flesh and bile samples were either non-detectable or just above the detection limit, and detectable levels of EROD activity occurred in 2 of 5 samples in one Alberta study. Consequently, chlorophenols and EROD are considered inadequate for use as tracers in future studies at these sites.

POSTER PRESENTATIONS/SÉANCES AFFICHES

ECOLOGICAL RISK ASSESSMENT OF NEW SUBSTANCES UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA): EFFECTS ASSESSMENT

A.I. Caldwell, R. Sutcliffe, and R.A.F. Matheson. Environment Canada, Hull, PQ

As part of a pollution prevention management approach to toxic substances, the Substances New to Canada provisions in CEPA ensure no new substance is introduced into the Canadian marketplace before an assessment of its 'toxicity' has been completed. Consequently, the purpose of the New Substance assessment process is to determine whether or not substances are, or are suspected of being, 'toxic' as defined in Section 11 of CEPA. The determination of 'toxic' consists of integrating the assessment of potential exposure of a substance to humans and components of the environment with potential adverse effects on humans or the environment. This paper describes Canada's approach to the environmental effects assessment within the context of the new substances program. Determining adverse effects of a substance on the environment involves developing a toxicity profile, which indicates the type of effect and the seriousness of that effect on a given organism at a known concentration of substance. Effect concentrations can be based on test data, or predications using structure-activity relationships (SAR), and consider environmental factors which may reduce or enhance the inherent toxicity of a substance. Application of assessment (or safety) factors to the effect concentration provides a concern concentration (CC) or chronic no-effect level. The subsequent ecological risk assessment is conducted using a quotient method comparing the concern concentration with predicted environmental concentrations. Adverse effects on the abiotic components of the environment are also considered including consequences such as depletion of the ozone layer and global warming.

ECOLOGICAL RISK ASSESSMENT OF NEW SUBSTANCES UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA): EXPOSURE ASSESSMENT

R. Sutcliffe, A.I. Caldwell and R.A.F. Matheson, Environment Canada, Hull, PQ

As part of a pollution prevention management approach to toxic substances, the Substances New to Canada provisions in CEPA ensure no new substance is introduced into the Canadian marketplace before an assessment of its 'toxicity' has been completed. Consequently, the purpose of the New Substance assessment process is to determine whether or not substances are, or are suspected of being, 'toxic' as defined in Section 11 of CEPA. The determination of 'toxic' consists of integrating the assessment of potential exposure of a substance to humans and components of the environment with potential adverse effects on humans or the environment. This paper describes Canada's approach to the environmental effects assessment within the context of the new substances program. The potential for exposure to a substance depends on the amount of substance relased into the environment and its environmental fate. The exposure assessment, therefore, consists of predicting the environmental concentrations (PEC) of a substance for releases resulting from its production, processing, uses, and disposal, and its environmental fate evaluated on the basis of intrinsic physical/chemical properties, environmental mobility, compartmentalization, and persistence. The assessment considers the use of the substance identified by the notifier, as well as other possible ways that substances might be used. The subsequent ecological risk assessment is conducted using a quotient method comparing the concern concentration with predicted environmental concentrations.

AN OVERVIEW OF THE GUIDANCE MANUAL ON ECOLOGICAL RISK ASSESSMENTS OF PRIORITY SUBSTANCES CONDUCTED UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT

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This poster provides an overview of the ecological risk assessment framework for assessments of priority substances in Canada, as presented in the guidance manual prepared by staff of the Commercial Chemicals Evaluation Branch, Environment Canada. The guidance manual promotes consistency in assessing priority substances and presents our assessment approaches to interested parties. Ecological risk assessment of priority substances involves three major steps: problem formulation, analysis and risk characterization. Problem formulation focuses on scoping and planning the assessment of the priority substance and identifies an assessment approach for the substance. The analysis step consists of three major parts: entry, exposure and effects characterization and represents the in-depth critical review of scientific information on the priority substance. Risk characterization uses the results of the analysis step to determine the likelihood and magnitude of risk by summarizing and describing these results (*i.e.*, CEPA toxic or not) to risk managers and others. The manual will be revised as needed, based on experience gained in the assessment of the 25 substances currently on the Priority Substances List.

PROBABILISTIC RISK ASSESSMENT OF PESTICIDE RESIDUES FOUND IN THE SURFACE WATERS OF ONTARIO AND QUEBEC

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Risk to aquatic organisms from exposure to residues of both individual and mixtures of pesticides found in the surface waters of Ontario and Quebec were assessed using a probabilistic approach as recommended by the SETAC Aquatic Risk Assessment Dialogue for Pesticides. Exposure characterization concentrated on monitoring data from Ontario and Quebec with focus on high use areas. Monitoring data containing mixtures of similar organophosphorus compounds were normalized to the concentration of the most toxic pesticide, azinphos methyl. The points upon which the normalization was based were the 10th centile of the toxicity distributions for all organisms. The azinphos methyl toxic equivalents were then added together and the total TE's plotted against the sensitivity distribution for azinphos methyl. Toxicity data were collected from the acute EPA One-liner database. Toxicity distributions were plotted using the same methods as the exposure data. The results of the risk assessments revealed that, generally, in cases where only a single pesticide was detected at a specific location, residues do not pose a significant risk to the aquatic environment. However, a small number of surface waters may have an increased risk due to mixtures of pesticides exhibiting the same mechanism of action present at the same time at a specific location For these locations, site-specific risk assessments along with further sampling should be conducted in order to fully assess the acute risks of pesticide exposures to the aquatic environment.

RISKS ASSOCIATED WITH METHYLMERCURY ACCUMULATION IN FISH FROM A RESERVOIR AND TWO LAKES IN BRITISH COLUMBIA

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Increased levels of mercury in hydroelectric reservoirs have posed a particular environmental problem in Canada. Fish in these aquatic systems accumulate unusually high levels of methylmercury in their tissue. This phenomenon has been attributed to several factors. Flooding of soil adds both inorganic mercury and organic substrate to the water. The increased level of mercury along with the microbial methylation stimulated by the input of organic nutrients results in higher concentrations of bioavailable methylmercury in the water. Fish are able to accumulate high levels of the toxin because they are relatively resistant to its effects. We have analyzed the relationship between size and methylmercury tissue levels for fish from a reservoir and two natural lakes in British Columbia. In all cases, we found a positive relationship between fish weight and methylmercury concentration in rainbow trout. We incorporated these results into a probabilistic assessment of exposure to methylmercury, and compared the predicted exposures to current Canadian reference doses. We extended the analysis by combining the probabilistic exposure assessment with a dose-response assessment of methylmercury's developmental neurotoxicity. In this way we were able to calculate potential risks to the children of women consuming fish from these bodies of water.

RISK TO BABIES AND FETUSES OF INUIT MOTHERS CONSUMING COUNTRY FOODS CONTAMINATED WITH CHLORINATED HYDROCARBONS

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This study provides an analysis of risk to Inuit fetuses and infants exposed to several chlorinated organic chemicals (COC) through placental transfer or breastfeeding respectively. The COC are DDT, Chlordane (CHL), hexachlorocyclohexane (HCH), polychlorinated camphenes (PCC), hexachlorobenzenes (HCB), PCB, and Dioxins (TCDD), and their respective environmental fates were distinguished between northern and southern Eastern Arctic regions (NEA and SEA). The evaluation considers toxicant-specific and - combined risks. The risk determinations are achieved through a computer-based model that accounts for known contaminant levels in country foods (e.g. whales, seals, caribou, etc.) maternal ingestion rates of country foods, diet and dietary habits. Various toxicokinetic factors taken from the literature were applied to derive breast milk, blood, and adipose tissue levels. Information on age related suckling rates, first year growth rates were part of the estimates of daily intake of the pollutants by breastfeeding children. The latter determinations were compared to maximal acceptable daily intake rate (ADI) criteria to derive a measure of risk. For fetal risk determinations, only the third trimester was considered. This decision was based on the epidemiological information that indicate health issues in Inuit children are of a nature associated with disruptions of neurological and other possible events of late gestation. A single placental transfer factor (PTF) was used to estimate fetal blood levels of the seven COC. Insufficient information was available to allow the QSAR-based determinations of contaminant-specific PFT's. Nevertheless, recently reported levels of PCB in cord blood of Quebec newborns permitted a validation check of this class of COC. The risk determinations for both fetuses and breast feeding infants show that there is reason for developmental health concerns for at least some of the COC's operating independently and for all the COC's acting jointly.

ANALYSIS AND RISK ASSESSMENT OF PRODUCTS MANUFACTURED WITH RECYCLED PESTICIDE CONTAINER PLASTIC

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Plastic pesticide container recycling has become an attractive disposal option because it is a valuable

source of resource recovery and generates marketable products. Because of the expenses involved with containers processing (washing or visual inspection), which are used to reduce residues, recycling has not been economically feasible on a large scale. As most collected containers are rinsed, it could be possible to produce safe products without extensive processing. This assessment evaluates fence posts made from Saskatchewan container plastic which has not undergone a washing process. Dislodgeable pesticide residues from the surfaces of the posts were analysed. Any remaining pesticides were drained from the containers prior to shredding and the material contained no free liquid. Other processed plastics were recycled into posts for comparative purposes; inspected Ontario/Quebec and Maritime plastics, and washed Saskatchewan plastic. The pesticides examined were 2,4-D isooctyl ester, trifluralin, lindane and chlorpyrifos. Two exposure scenarios were assessed; dislodgeability from 100 cm^2 for 15 min. (casual contact), and 200 cm^2 for 8 h (occupational contact). Dislodged residues were compared with the acceptable daily intakes (ADI's) for the individual pesticides. ADI's were not exceeded for either exposure scenario for any post. However, the ADI for chlorpyrifos was approached from simulated occupational contact and overall variability was high. Recycled products made from container plastics examined in this study should not pose an unacceptable risk. Exposure would be significantly minimized by wearing protective gloves, which should be advised for occupational contact. Quality control measures can also be taken to ensure the consistent safety of the recycled products.

ENVIRONMENTAL EFFECT MONITORING PROGRAM: A CANADIAN EXPERIENCE

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Environmental Effects Monitoring (EEM) program is an essential element to an efficient pollution control program. While regulations and water quality criteria provide numerical guidance on environmental protection, an EEM program provides a feedback mechanism to measure the performance of the regulations and the criteria used in a pollution control program.

In 1991, the Canadian federal government initiated an EEM program based on an industrial-sector approach. The government of Canada has applied the approach to the monitoring of the Pulp and Paper Mill effluents and is developing its application to the metal mining industries. Canada is the first country to integrate an EEM approach into its regulatory regime and, as preliminary results indicate, has proven that the approach is implementable from a regulatory, management and scientific point of view. This poster presents the main components of the program as it exists in Canada. It describes the EEM Program as a whole, the Pulp and Paper and the Aquamin Programs and the Information Management structure of the Environmental Effects Monitoring program. The emphasis is put on the role of EEM as a component of solution to pollution control measures.

MONITORING OF PHYSIOLOGICAL RESPONSES OF FISH TO PULP MILL EFFLUENTS IN AN ESTUARINE ENVIRONMENT

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An ongoing five-year study (1992-97) is being conducted under the Department of Fisheries and Oceans Toxic Chemicals Green Plan Program to assess the effects of pulp mill effluents on resident and laboratory-reared fish. In the spring (prespawning) and autumn (postspawning) of 1995, work was conducted on two rivers in New Brunswick to increase our understanding of the effects of pulp mill effluents in estuarine environments. *Fundulus heteroclitus* (mummichog) are resident in rivers in this

area and have a sedentary nature, making them particularly useful in correlating impacts of mill effluent with resident fish. While two localities on the Bouctouche River served as reference sites, the study river was the Miramichi, which receives pulp mill effluent. Three sites were established on the Miramichi River, in varying proximities to the mills. Mummichog were captured at all five locations on the two rivers and MFO and steroid analysis were conducted. Laboratory effluent tests were also conducted to assess MFO induction and steroid hormone depression occurred at effluent concentrations up to 50%. Wild fish showed the highest MFO activity closest to the pulp mills, but significant seasonal changes were seen, and induction was evident but lower at the reference site. Physiological responses were more difficult to interpret and although some changes were seen, they were more difficult to relate to effluent exposure. Recommendations will be made for monitoring effluent impacts on estuarine fish.

COMPARISON OF SUBLETHAL TOXICITY IN LIQUID EFFLUENT FROM TWO PULP AND PAPER MILLS DISCHARGING TO THE MIRAMICHI RIVER ESTUARY, NEW BRUNSWICK

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Sublethal toxicity of liquid effluent of two pulp and paper mills discharging to the Miramichi River Estuary was assessed, as part of the First Cycle of the federally regulated Environmental Effects Monitoring Program. This poster presents the results of the quarterly sublethal bioassays of samples taken from each facility in 1994 and 1995. Changes in liquid effluent treatment are described, as one facility brought secondary treatment on-line during the sample period. Opportunities for obtaining time series data on trends in sublethal toxicity from two different pulp and paper processes, as well as from two different secondary treatment systems, each discharging to the same receiving water body, are presented.

Repap New Brunswick Inc. operates a 1500 ADMTPD (reference production rate) bleached kraft pulp and coated paper mill on the north shore of the Northwest Miramichi River, near its confluence with the Southwest Miramichi River. The Company also operates a 360 ADMTPD (reference production rate) groundwood pulp mill on the south shore of the Southwest Miramichi River, near its confluence with the Northwest Miramichi River. Liquid effluent from the kraft mill is subject to secondary treatment in an aerated lagoon before discharge. The groundwood mill effluent has been subject to secondary treatment in a sequential batch reactor system since December, 1995. Prior to this the effluent received primary treatment.

Results of the inland silverside early life development, sea urchin fertilization and *Champia* development sublethal bioassays of samples taken quarterly over a one year period at each facility are presented. The kraft mill showed changes in sublethal toxicity over the year of sampling, although the trend in these changes was not consistent between the three tests. The groundwood mill effluent showed similar seasonal trends, as well as differences between the three tests. The groundwood mill effluent showed dramatic improvement in sublethal toxicity by the inland silverside bioassay, after the secondary treatment came on-line. Some improvement in toxicity was shown with the sea urchin test, while no change was noted with the *Champia* test.

TREE SWALLOWS AS BIOINDICATORS OF RIPARIAN WILDLIFE EXPOSURE TO PULP AND PAPER MILL EFFLUENT

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Pulp mill effluent may add nutrients and toxins to receiving waters. Aquatic biota in receiving waters are often affected from exposure to such effluent. Predominant effects include cultural eutrophication and physiological and ecological effects on fish. If these effects are mediated or transferred through food chains, then riparian wildlife that feeds on insects emerging from pulp mill receiving waters may be similarly affected. This study examined MFO activity (ethoxyresorufin-O-deethylase, EROD), highly carboxylated porphyrins (HCP's), plasma 17*B*-estradiol and reproductive performance of a riparian species, the tree swallow, upstream and downstream from two bleached kraft mills in the Canadian prairie provinces. Both mills use modern bleaching techniques and have aerated secondary treatment ponds. Riverine insects comprised 50-60% of the tree swallow diet at the two study areas. We did not detect any effects on EROD activity or HCP concentrations in 16-day old nestlings that were consistent with a pulp mill effect. In 1995, 17*B*-estradiol was slightly lower in incubating females downstream from one of the mills than it was in females at upstream sites. At both study areas, there were no among-site differences in reproductive performance consistent with a pulp mill effect after accounting for ecological variables known to affect reproductive performance. However, 16-day old chicks downstream from both mills were slightly larger compared to their counterparts at upstream sites. Work is being done in 1996 to further investigate the lower estradiol in incubating tree swallows downstream from one of the mills.

USING CAGED BIVALVES TO ASSESS EXPOSURE AND EFFECTS: CASE STUDIES AND POSSIBLE APPLICATIONS AT PULP AND PAPER MILLS

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There is general agreement between regulatory agencies and industry that new approaches are necessary to monitor and assess environmental conditions in the vicinity of pulp and paper mill effluents. Current field monitoring approaches utilize benthic invertebrates and fish to estimate exposure and effects. The problems inherent with these approaches include [1] availability of appropriate species, and [2] difficulty in interpreting effects and exposure data. *In situ* studies with caged bivalves facilitate measuring bioaccumulation and bioeffects because [1] they are easy to collect, cage, and measure, [2] they will accumulate bioavailable chemicals, and [3] they can be measured for adverse effects. Caging provides a unique opportunity to make synoptic measurements of exposure and effects; caging also permits making these measurements over space and time. Over the past five years the design and methodology has been refined to include mussels, clams, and oysters; marine, estuarine, and freshwater habitats, and depths up to 30 m. The historical use of caged bivalves in aquatic monitoring programs will be reviewed. Examples of the various deployment configurations will be presented, and results from long-term studies where exposure and effects data were used to identify temporal and spatial trends, site-specific differences, contaminant sources, and dose-response relationships. In addition, results will be presented from a recent caged mussel study conducted in the Pacific Northwest to evaluate conditions in the vicinity of effluents discharged from a pulp and paper mill.

AQUATIC QUALITY MONITORING NAHANNI NATIONAL PARK RESERVE, NT

D. Halliwell. Environment Canada, Yellowknife, NT.

Environment Canada and the Parks Canada have monitored water, sediment, and fish tissue quality in Nahanni NPR since 1988 as an intensive (1988-91) study and as five year (1992-97) follow-up

monitoring program. The initial study focused on characterization of water quality at five of the 13 sites studied within the Park, documenting seasonal variability and setting long-term and short-term objectives (LTOs, STOs) for 28 water quality variable. The more recent monitoring refines initial water quality objectives at five sites using a larger dataset collected for seven years over a wide range of river flows. The monitoring program will also establish additional objectives for water quality at two new sites, and sediment quality at one site. Spatial and temporal variability (both seasonality and trend analyses) are dealt with, and values are compared with Canadian Water Quality Guidelines and (Draft) Canadian Sediment Quality Guidelines. In related 1992 and 1994 studies, DFO assessed with fish tissue sampling and analyses at two sites. Aquatic quality baseline monitoring is justified within this portion of the Tundra Cordillera Ecozone because the NWT's most visited national park is downstream from the Tungsten (W-Cu) and Cadillac (Ag-Pb-Zn) mines and dozens of undeveloped mineral occurrences/mining claims described in a recent GSC Open File. The Cadillac Mine area in Prairie Creek is actively being explored/developed and an environmental impact assessment currently underway.

ORGANOCHLORINE CONTAMINANTS IN FISH FROM THE FRASER RIVER BASIN

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The Environmental Quality component of the Fraser River Action Plan is assessing condition and health of resident fish in the mainstem Fraser and major tributaries. As an indicator of contaminant levels in fish in the basin, peamouth chub (*Mylocheilus caurinus*) and mountain whitefish (*Prosopium williamsoni*) were sampled and measured for levels of a wide range of organic and metal contaminants in tissues. Both chub and whitefish were collected from 4 reaches on the Fraser River (upstream of Prince George (near Hansard), downstream of Prince Greorge (and Woodpecker), downstream of Quesnel (at Marguerite), and near Agassiz), on the Nechako River, and in two reaches of the Thompson River system (North Thompson near McLure and Thompson River downstream of Kamloops Lake). Peamouth chub were also sampled at 4 additional reaches in the lower Fraser. Preliminary analysis of data from the 1994 sampling show generally low levels of contamination, with patterns of contamination related to upstream effluent sources. Contaminants in fish from reaches in the Thompson system suggest these populations may be quite uniform, and that the 'resident' fish of the North Thompson travel some considerable distances.

PROSPECTING FOR ORGANOCHLORINE COMPOUNDS IN YUKON WETLANDS USING WOOD FROGS (*Rana sylvatica*)

G. Whitley, P. Roach and M. Palmer. Indian and Northern Affairs Canada, Whitehorse, YK.

The Arctic Environmental Strategy has provided the resources to identify and cleanup some contaminated sites in the Yukon. Resident fish, birds or mammals are not always available or suitable for environmental impact assessment. The wood frog (*Rana sylvatica*) is a common resident of boreal forest wetlands. Where wetlands are adjacent to known or suspected contaminated sites or where DDT may have been used for mosquito control, the wood frog could be a suitable indicator for environmental contamination. Wood frogs were captured from two wetlands in Whitehorse and submitted for analysis. Two frogs from a pond where DDT was used for mosquito control were found to contain *p,p'*-DDE (31 and 25 ng/g) while two frogs from another pond where DDT was likely not used contained little *p,p'*-DDE (2 and 4 ng/g). Other organochlorines were not detected. Frogs from wetlands suspected of being contaminated with DDT and PCB's have been captured and submitted for analysis. Preliminary results will be presented. It is hoped that wood frogs will prove useful as a prospecting tool for initial

assessment of suspected contaminated sites.

MERCURY AND OTHER METALS IN WATER, SEDIMENT AND FISH FROM A CONTAMINATED ECOSYSTEM AT DISCOVERY MINE, NT

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Giauque Lake has received considerable attention from government environmental investigators since ecological studies in 1977 indicated that mercury contamination of the ecosystem was a direct result of effluent discharges from Discovery Mine's operations between 1945 and 1969. Other reasons are related to the potential impact of mercury on the ecosystem such as commercial fisheries and water use in adjacent downstream lakes. The purpose of the study was to re-evaluate the extent of mercury contamination in the lake system since the last ecosystem assessment performed in 1978. The study involved an examination of several ecological compartments - water, suspended solids, fish species and sediment cores. The concern was whether the present level of mercury contamination is due to the continual erosion of the tailings or a result of past discharges of toxic effluent during the operation of Discovery Mine.

Results from the analysis of mercury levels in the tailings run-off and in the suspended sediment in the water column indicate that the physical processes occurring at the tailings fan (whether they be rainfall, snowmelt, wind or erosion by wave action) have a significant impact on the overall metal loadings to Giauque Lake. In comparison with previous results, the examination of the surficial layer indicates that the deposition of particulate mercury is still occurring. Mercury in pore water was detected in heavily contaminated surficial sediments but represented less than 0.01% of the total mercury.

The analysis of mercury and other metals in the muscle and organ tissues of the 5 species of fish represents the most comprehensive survey to date. Mercury concentration levels in the fish species were compared with results from 1978 in an attempt to assess contaminant clearance from the ecosystem. Ratios of mercury in the liver, kidney and muscle for each fish species were assessed for mercury uptake toxicological differences. Based on the elevated levels of mercury in fish muscle, the Health Protection Branch has recommended consumption guidelines for all 5 species of fish from Giauque Lake and lake trout and northern pike from Thistlethwaite Lake.

OCCURRENCE OF ALKYLPHENOL ETHOXYLATE SURFACTANT METABOLITES IN SEWAGE EFFLUENTS AND SLUDGE

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Nonylphenol polyethoxylates and its refractory metabolites are on the second Priority Substances List (PSL2) to determine if they are 'toxic' as defined under the Canadian Environmental Protection Act. This study addresses the need for data on their occurrence in sewage effluents and sludge in Canada. Samples of effluent and sludge were collected from 20 wastewater treatment plants across Canada. These samples were analyzed for 4-nonylphenol (4-NP), nonylphenol ethoxylate (NPE_1), nonylphenol diethoxylate (NPE_2) and 4-*tert*-octylphenol (4-t-OP). Measurable quantities of these chemicals were found in almost all primary influent and sludge samples. In the primary influent, concentrations ranged from <0.005 to 21 µg/L for 4-t-OP, from 1.1 to 156 µg/L for 4-NP, from 0.75 to 43 µg/L for NPE_1 and from 0.033 to 24 µg/L for NPE_2. Sludge concentrations (based on dry weight) ranged from < 0.010 to 20 mg/kg, from 8.4 to 850 mg/kg, from 3.9 to 437 mg/kg and from 1.5 to 297 mg/kg for 4-t-OP, 4-NP,

NPE_1 and NPE_2, respectively. Of the final effluent samples, only 50% contained any 4-t-OP and concentrations ranged from < 0.005 to 0.37 µg/L. However, 90% or more of the final effluent samples had detectable levels of 4-NP, NPE_1 and NPE_2. The 4-NP concentrations varied from < 0.020 to 13 µg/L, NPE_1 ranged from < 0.020 to 43 µg/L and NPE_2 was found in the range of < 0.020 to 33 µg/L.

POTENTIAL TOXICITY OF AN AQUATIC HERBICIDE IN A LITTORAL ZONE

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In Alberta 'Reglone A' (active ingredient = diquat) is used to control submerged and free-floating vegetation and algae mostly in ornamental lakes and dugouts. The available scientific data on the toxicity of diquat show that it is less toxic to fish than invertebrates. An approval for the restricted use of 'Reglone A' was given to a summer village at Lac Ste. Anne. The herbicide was applied in preselected areas to reduce vegetation for swimming and boating access. A field study was designed to determine the toxicity of 'Reglone A' on three invertebrates that live and feed on the aquatic vegetation, bottom sediment and the water column of the lake. Laboratory experiments were conducted to determine the modifying effect of sediment and vegetation on the toxicity of the herbicide to *Daphnia magna* (Crustacea). The results of the field study showed a statistically significant decrease in the survival of *Gammarus lacustris* (Crustacea), but the survival of *Lymnaea stagnalis* (Mollusca) and *Agrypnia* sp. (Insecta: Trichoptera) were not affected. Toxicity on *D. magna* was reduced by either the vegetation (*Lemna minor*) or artificial substratum (quartzite). This result demonstrated the processes that probably reduced the toxicity of 'Reglone A' under field conditions. The field results showed an effect on the most sensitive taxon (Crustacea), that is important in the diet of fish and ducks. Toxicity of aquatic invertebrates can, therefore, occur at concentrations of diquat normally used in field applications and caution should be used when using 'Reglone A' to control vegetation.

TOXICITY OF FREE-WAY STORM WATER

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ABSTRACT

Storm water and melted curb snow, collected from an urban free-way, were tested for toxicity. Toxicity tests included acute lethality to rainbow trout (*Oncoryhnchus mykiss*) and *Daphnia magna*, reproduction impairment with *Ceriodaphnia dubia*, and Mixed Function Oxidase [Ethoxyresorufin- *O*-deethylase (EROD)] induction in rainbow trout. Free-way storm water and snow samples were, from time to time, lethal to trout and *Daphnia* and reduced reproduction of *Ceriodaphnia*. Invertebrate lethality and reproduction impairment was associated with elevated levels of Chloride. Elevated levels of Zn and Cu were also potentially lethal to trout and invertebrate species. In addition, juvenile trout exposed for 96 h experienced a significant induction of EROD enzymes. Possible causes for this EROD induction have been studied. A retention pond was found to reduce the lethality and the potential for EROD induction in free-way storm water.

INTRODUCTION

Modern free-ways occupy vast areas of impervious surfaces which discharge large volumes of storm water, over a short time interval, to adjacent water bodies. Free-way surfaces collect contaminants from vehicular traffic. In Ontario, traffic densities can reach 350,000 vehicles per day (MTO, 1992). Highway

storm water and downstream sediments have been shown to contain elevated levels of Zn, Cu, Pb, Cr and Hg (Moxness, 1986; Driscoll et al., 1990; Maltby et al., 1995). Organic contaminants such as PAHs also accumulate in sediments downstream of highways (Maltby et al., 1995). Contaminant loading appears to be a function of highway size and traffic density (Moxness, 1986; Driscoll et al., 1990). An additional contaminant is road salt, used extensively in winter to deice highway surfaces.

While impacts of such contaminants on aquatic organisms are predicted, relatively few studies have examined their effect on aquatic communities or the toxicity of highway storm water in laboratory bioassays. This study used biological tests to assess the toxicity of free-way storm water and attempted to relate toxicity to levels of specific contaminants. The study also examined the effects of a retention pond in modifying the toxicity of free-way storm water.

METHODS

Sampling

Free-way storm water (100 L) samples were collected from a storm sewer by automated sampler initiated on water flow (Fig. 1). When necessary, a grab sample was collected, using a stainless steel bucket, from the sewer well at the same sewer location. As an alternative sampling technique, storm water samples were collected in a barrel set below a bridge down spout (Fig. 2). In winter, snow samples, to make 100 L water, were collected from the ploughed snow bank (Curb snow) within 3 m of the free-way. As a control, "Clean" snow was collected from the surface of snow bank a minimum of 100 m from free-way. Snow samples were thawed and adjusted to 15°C before testing. Free-way storm water samples were collected before and after passage through a storm water retention pond. Samples (100 L) were collected by automated sampler at the pond's storm water inlet sewer and at the pond's outlet sewer. For toxicity testing, samples were transported to the laboratory in 20 L buckets lined with food-grade polyethylene bags. For organic chemical analysis, samples were collected in stainless steel canisters and transported to the laboratory in the canister or in glass bottles.

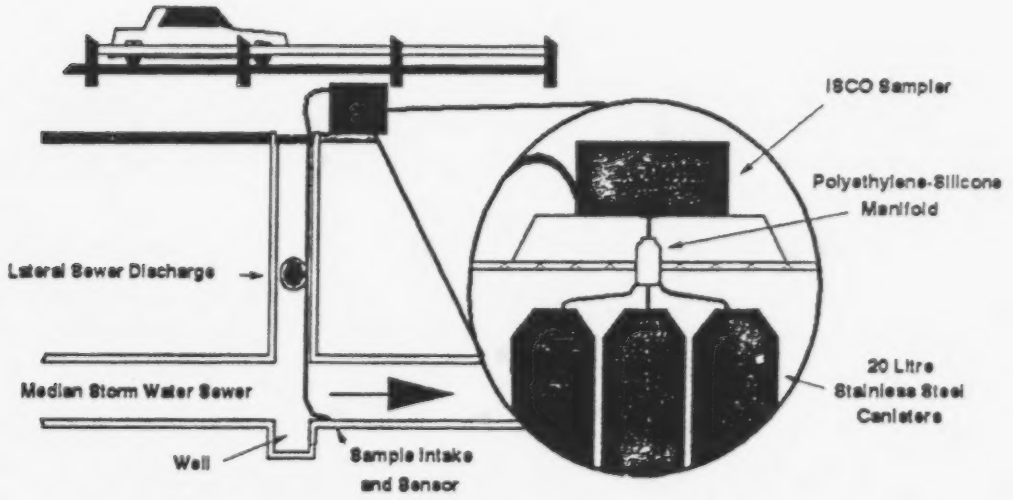

Fig. 1. Sampling of a free-way storm water sewer.

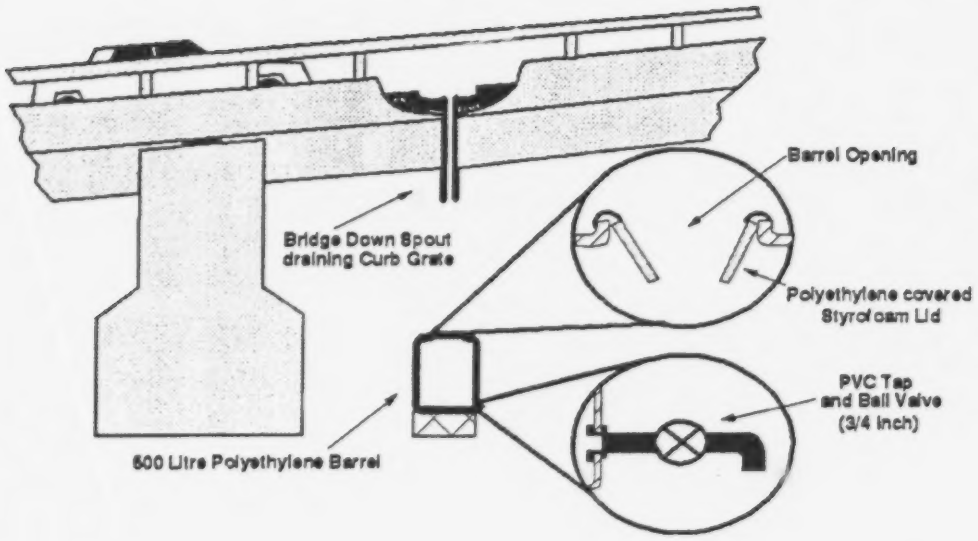

Fig. 2. Sampling of storm water at free-way bridge down spout.

Toxicity Tests

Toxicity tests were performed in the laboratories of the MOEE Standards Development Branch, Aquatic Toxicology Section, 125 Resources Road, Etobicoke, ON. Bioassays included rainbow trout (*Oncoryhnchus mykiss*) 96 h acute lethality test, *Daphnia magna* 48 h acute lethality test, and the *Ceriodaphnia dubia* 7 d reproductive impairment test, each by their respective Canadian protocol (Environment Canada, 1990a; Environment Canada, 1990b; Environment Canada, 1992).

Rainbow trout 96 h liver microsomal EROD induction was measured by an MOEE internal protocol. Fish were exposed for 96 h to the highest non-lethal dilution of the water sample. As a control, fish were exposed to laboratory dilution water (Toronto dechlorinated tap water) for the same time period. At the end of exposure, fish were sacrificed and their excised livers were pooled. Ethoxyresorufin-*O*-deethylase (EROD) activity was measured in the liver 100,000 x g microsomal fraction by the method of van den Heuval (1990). The intensity of response was expressed as EROD induction calculated by dividing the EROD Specific Activity measured in exposed fish by that of dilution water control fish.

Chemical Tests

All chemical tests were performed by the MOEE, Laboratory Services Branch, 125 Resources Road, Etobicoke, ON. Chemical tests included [1] water quality parameters in the mg/L range for pH, conductivity, hardness, alkalinity, particulate residue (RSP), Total residue, DIC, DOC, BOD, Ca^{++}, Mg^{++}, Na^{+}, K^{+}, $F\Gamma$, Cl^{-}, $SO_4^{=}$, TKN, NH_4^{+}+NH_3, NO_3^{-}, NO_2^{-} and including Total CN^{-}, Free CN^{-}, [2] metals in the mg/L range and reported as Total metal (soluble plus complexed) for Cu, Ni, Pb, Zn, Fe, Cd, [3] methylene chloride extractable material (SOLSX'r) expressed as weight in mg/L, [4] organic compounds including PAHs, phthalates, chloroalkylethers, aromatic ethers, nitrotoluenes, chlorinated phenolics, nitrated phenolics measured in the µg/L range, and for pesticides, chlorinated organics, total PCB and

Table 1. Toxicity of highway storm water and snow samples.

Species	Result	Numbers of samples				
		Snow		Free-way storm water	Free-way rentention pond	
		100m free-way	curb at free-way		Inlet	Outlet
Rainbow trout 96 h LC_{50}	Non-lethal	4	3	9	10	11
	LC_{50} >100%	0	1	1	2	1
	LC_{50} <100%	0	0	7	2	0
	Total	4	4	17	14	12
D. magna 48 h LC_{50}	Non-lethal	4	1	10	15	14
	LC_{50} >100%	1	2	4	2	1
	LC_{50} <100%	0	1	2	0	0
	Total	4	4	16	17	15
C. dubia Reproduction 7 d EC_{50}	Non-toxic	0	0	7		
	EC_{50} >100%	0	0	2		
	EC_{50} <100%	1	2	6		
	Total	1	2	14		

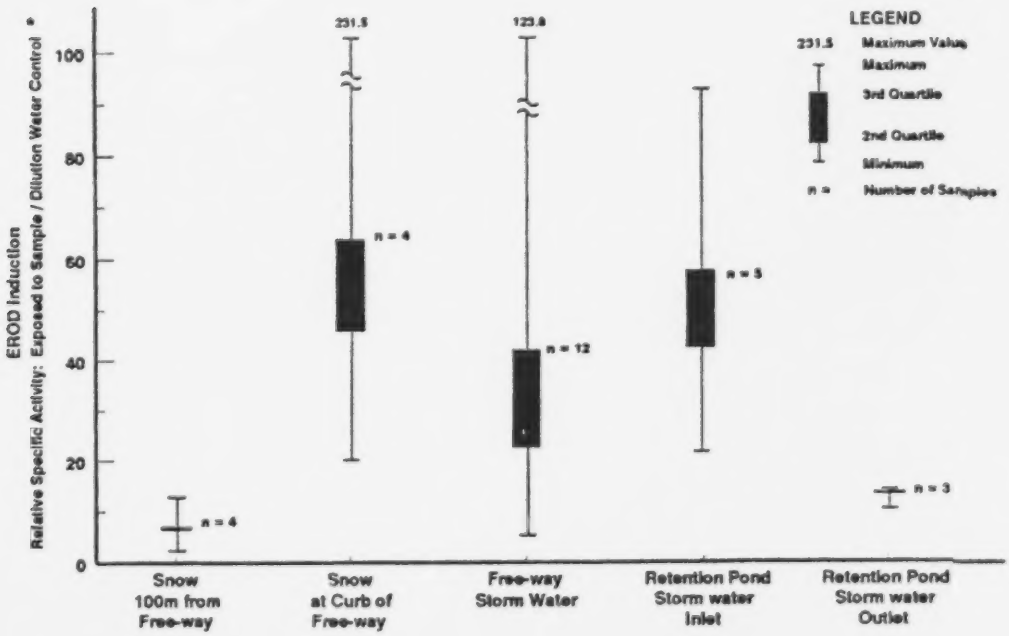

Fig. 3. EROD induction in rainbow trout exposed to storm water and snow samples. *Control EROD specific activity = 3.58±1.17 pmoles/min/mg microsomal protein.

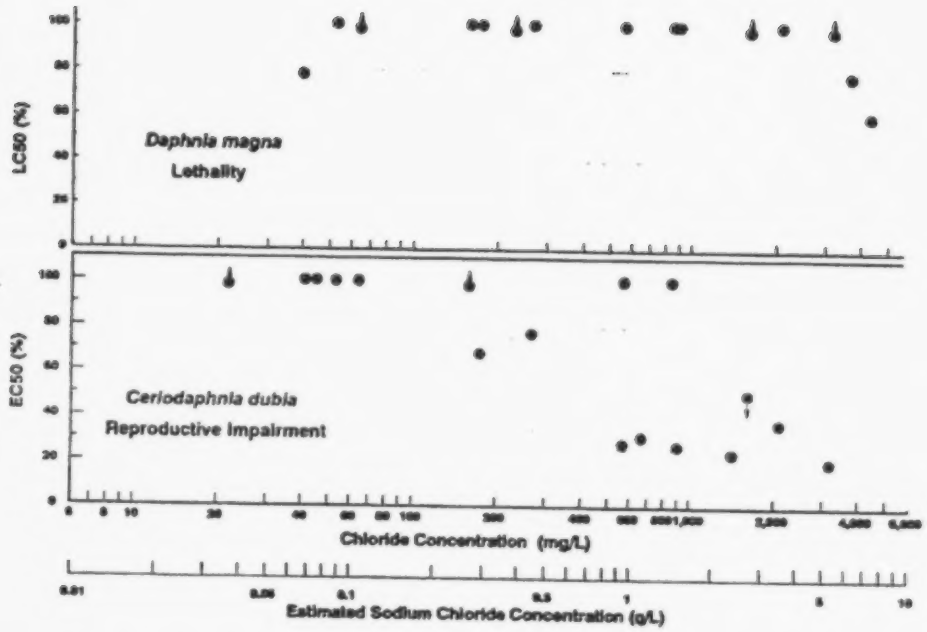

Fig. 4. Relationship between storm water chloride levels and toxicity to invertebrate species. ↑ indicates greater than 100%. ↓ indicate less than plotted value.

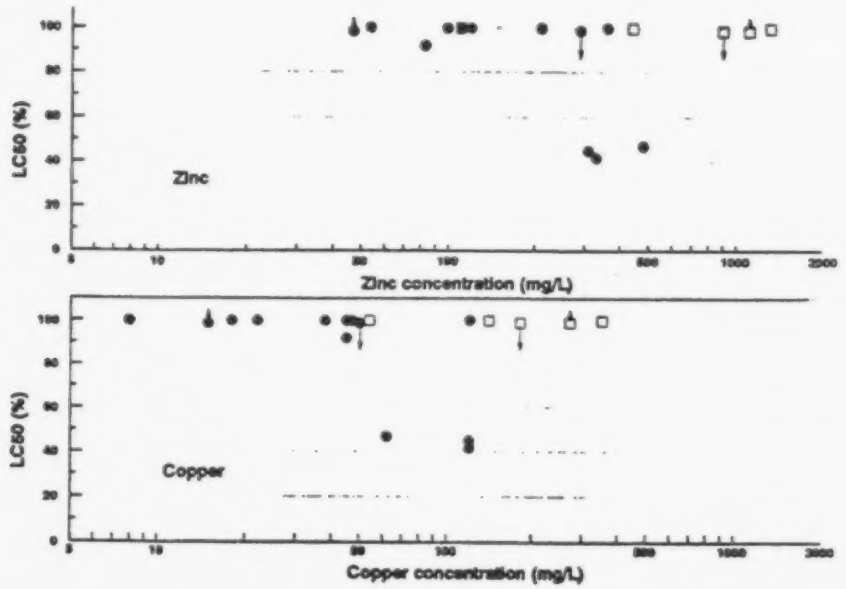

Fig. 5. Relationship between free-way water zinc and copper levels and lethality to rainbow trout. ● RSP < 100 mg/L; □ RSP > 100 mg/L; ↑ greater than 100&; ↓ less than 100%.

Mirex measured in the ng/L range, and [5] Gas Chromatography/Mass Spectroscopy characterization of organic compounds.

RESULTS

Free-way storm water was lethal to rainbow trout and *D. magna*. Lethality to rainbow trout was measured in 8 of 17 samples, 7 of these with a LC_{50} <100% (Table 1). Six of 16 samples were lethal to *D. magna*, with two at LC_{50} values <100%. In addition, 8 of 15 samples impaired reproduction of *C. dubia* with 6 samples at ED_{50} (50% reduction in neonates) less than 100%. All but one of the snow samples were non-lethal to rainbow trout (Table 1). In contrast, curb snow was lethal to *D. magna* (in 3 of 4 samples) and impaired reproduction of *C. dubia* (in both samples tested). A limited number of tests detected invertebrate toxicity in snow 100 m from the free-way. *D. magna* lethality was detected in 1 of 4 samples. The single sample tested impaired reproduction of *C. dubia*.

Free-way storm water and curb snow samples were an effective inducer of EROD. Storm water induced rainbow trout liver EROD levels an average of 30, with an extreme of 124 times higher than levels in control fish held in dilution water (Fig. 3). EROD induction by curb snow samples averaged 55, with an extreme of 231.5 times higher than controls. Snow samples taken 100 m from the free-way generally resulted in less than 10 fold EROD induction.

Retention of storm water in a holding pond reduced the incidence of toxic samples. The number of samples lethal to rainbow trout and *D. magna* at the pond inlet was reduced at the pond outlet (Table 1). EROD induction was reduced from levels averaging 50 fold at the pond inlet to levels of 10 fold at the outlet and comparable to that of snow samples collected 100 m from a free-way (Fig. 3).

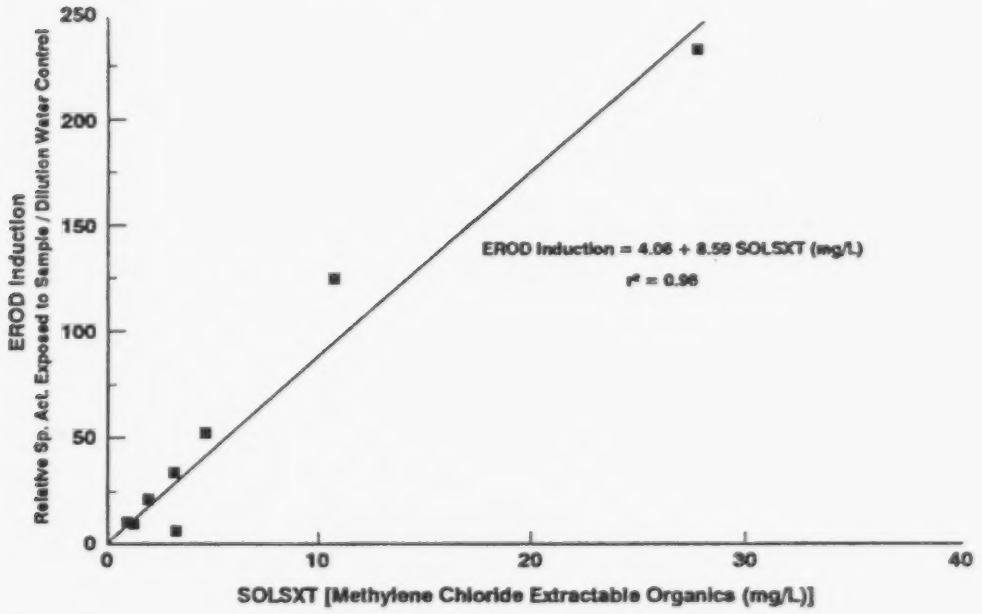

Fig. 6. Relationship between SOLSXT (methlylene chloride extractable organic) content in free-way storm water an EROD induction in rainbow trout.

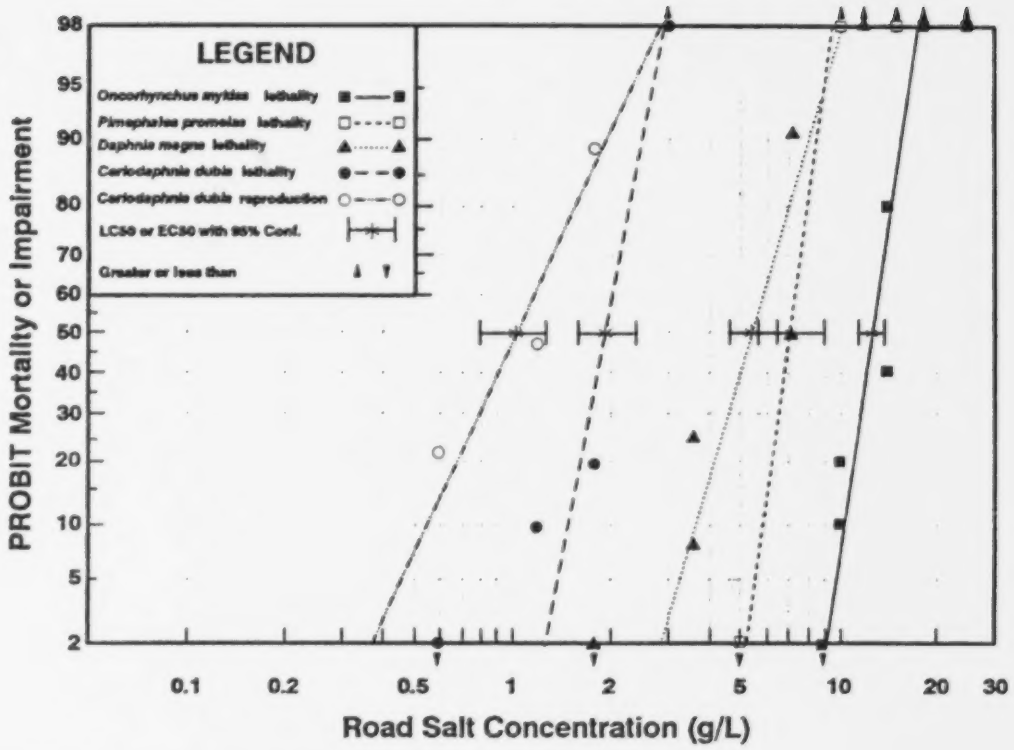

Fig. 7. Toxicity of road salt to aquatic species.

Chloride ion (Cl^-) concentration, principally from dissolved Sodium Chloride, related to invertebrate toxicity. Samples with Cl^- levels greater than 600 mg/L (estimated NaCl level >1 g/L) generally resulted in *C. dubia* reproductive impairment (Fig. 4). Storm water samples with Cl^- greater than 3000 mg/L (estimated NaCL >5 g/L) were lethal to *D. magna*. Lethality to *D. magna* (one sample) and reproductive impairment of *C. dubia* (two samples) at Cl^- levels less than 300 mg/L suggest other toxic contaminants. However, Cl^- content of storm water did not relate to lethality with rainbow trout.

Rainbow trout lethality was related to Zn or Cu concentrations, however, this relationship was confused in samples with RSP (particulate residue) levels greater than 100 mg/L (Fig. 5). When RSP was less than 100 mg/L, samples with 'Total' Zn >300 mg/L or "Total" Cu >50 mg/L were lethal to rainbow trout. Lethality to rainbow trout was reduced in samples with RSP greater than 100 mg/L, even if "Total" Zn and Cu levels exceeded 300 and 50 mg/L, respectively.

EROD induction was strongly related to SOLSXT (Methylene chloride extractable organic) content of storm water samples (Fig. 6). Although SOLSXT content and EROD induction were related, the causative compound or compounds have not been identified.

CONCLUSIONS

Approximately one half of the free-way storm water and snow samples caused effects in one or more bioassay. Sample lethality was detected with either rainbow trout or *D. magna* at LC_{50} values ranging from >100% (detection limit) to a sample LC_{50} less than 50%. Both rainbow trout and *D. magna*

bioassays were required to detect all lethal samples. These results support the need for a battery of bioassays to completely describe sample toxicity. *C. dubia* reproductive impairment detected toxicity in the highest frequency of storm water and snow samples. *C. dubia* was also the most sensitive species with EC_{50} values ranging from >100% to a sample ED_{50} of 20%.

Free-way storm water and snow samples were an effective inducer of EROD in the liver of rainbow trout. EROD induction, to levels 20 to 65 times that of control fish, were typically measured. These levels exceeded the EROD induction (1.3 to 19.5 times control) measured with pulp mill effluent (Gagne and Blaise, 1993; Burnison et al., 1996). When the potency of induction and volumes of storm water are considered, free-way run off may be a significant contributor to elevated EROD levels in feral fish in the adjacent aquatic environment, particularly in urban locations.

C. dubia reproductive impairment and *D. magna* lethality was related to the concentration of Chloride ion, which originate primarily from dissolved Sodium chloride. *C. dubia* reproductive impairment occurred with samples exceeding 1 g/L NaCl (Fig. 4) which was comparable to the ED_{50} (1 g/L) for road salt reference (Fig. 7). Similarly the NaCl content (5 g/L) of samples lethal to *D. magna* where comparable to the LC_{50} for road salt with this organism (Fig. 7). Rainbow trout was relatively resistant to road salt at a LC_{50} of 12 g/L, which exceeded the highest measured $Na^+ + Cl^-$ levels all samples (Fig. 4). Fathead minnow (*Pimephales promelas*) was not used in the study of storm water. However the LC_{50} (7 g/L) of road salt with fathead minnow, approached the highest estimated NaCl content of curb snow samples.

Storm water samples lethal to rainbow trout contained Zn and Cu levels exceeding 300 and 50 mg/L respectively (Fig. 5). However sample lethality and levels of Zn and Cu were not related in samples with particulate residue exceeding 100 mg/L. It was likely that the bioavailability of these metals was reduced in samples of higher particulate residue. These findings suggest predictions of sample toxicity based solely on measured 'Total' metal levels may be unreliable.

EROD induction by Free-way storm water and snow samples was related to the total Methylene chloride extractable organic content of these samples (Fig. 6). These findings suggest the responsible compound(s) were organic in nature. However, the specific contaminant(s) associated with EROD induction have not been identified.

Highway storm water retention ponds have been included in modern free-way design as a means of controlling the hydrological impacts of storm water runoff. In addition, these retention ponds have been shown to reduce the lethality and EROD induction potential of free-way storm water. Although further study of the action of retention ponds is necessary, it is likely that primary settling of insoluble metal complexes and particle associated EROD inducing substances as well as photo and/or biodegradation are mechanisms in the reduction of aquatic toxicity in these facilities.

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IDENTIFICATION AND TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN HIGHWAY RUNOFF

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Urban runoff is a potential source of polycyclic aromatic hydrocarbons (PAHs). Produced in the use of fossil fuels, and transported during rain/snow events, these compounds can have an effect on receiving waters. A study of highway runoff to determine levels of PAHs and their potential toxic effects was undertaken. Whole water samples were collected from a local highway bridge, and filtered through a 1 mm glass fibre filter. Filtered water and suspended sediment were each extracted with an organic solvent and the extracts were analyzed by gas chromatography/mass spectrometry (GC/MS). Individual PAH were identified and quantified with phenanthrene, fluoranthene, and pyrene predominant.

Toxicity testing of the sediment extracts in the Mutatox® mutagenicity test gave a mild toxic response. To further attribute the toxic effect to the identified PAHs, the sediment extract was fractionated by high performance liquid chromatography (HPLC) on an aminopropylsilane column which separates the PAHs on the basis of ring number and structure. Each fraction was then tested in the Mutatox® test and the PAH separation confirmed by GC/MS. Results indicate a toxic response in several HPLC fractions.

TOXICITY AND CONTAMINANTS STATUS OF SUSPENDED AND BOTTOM SEDIMENTS FROM URBAN STORMWATER PONDS

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ABSTRACT

A seasonal survey of four stormwater detention ponds in the Greater Toronto Area was conducted to assess the nutrient and metal contamination of suspended and bottom sediments and relate it to their ecotoxicological status. The toxic responses of sediments were evaluated using a battery of tests approach (Dutka 1988). This is a first study attempting to integrate the results of toxicological tests with the concentrations of contaminants in sediments in urban stormwater detention ponds.

The data indicate a widespread presence of bioavailable toxicants in the ponds studied. No noticeable decrease in toxic responses, nutrient and metal concentrations was observed between the inlets and outlets of these ponds. Comparable results were obtained from pore water tests, using Microtox, SOS-

Chromotest and the submitochondrial particle (SMP) tests. These tests indicated that Col. Sam Smith and Unionville ponds, which drain industrial and residential catchments, respectively, were more impacted than the other studied ponds. The suspended and bottom sediments of these ponds also had the highest heavy metals concentrations.

INTRODUCTION

Urban areas are important sources of the non-point source pollution. Stormwater detention ponds are one of several management options used to mitigate the impact of urban pollution on the downstream water quality. The newly constructed ponds were designed to provide partial treatment of urban stormwaters by capturing the sediments. The close association of contaminants with sediments has been well documented. Hence, removal of sediment associated contaminants, is the principal mechanism of pollution abatement in these ponds. Numerous such structures are in operation in the Greater Toronto Area (GTA).

Commonly, assessment of pollution in stormwater detention ponds was based on the chemical characterization of water and sediments (Marsalek et al., 1993; Mayer et al.,1996). However, chemical analysis cannot measure toxicity. They can only detect toxic substances and provide their quantitative estimates. Recently, Dutka et al. (1994a, 1994b) carried out an ecotoxicological study to assess pollution in stormwater detention ponds in the GTA. These studies provided information on the toxicity of suspended and bottom sediments in stormwater detention ponds, however, they did not provide data on the chemical composition of sediments or ambient limnological conditions in the ponds. To augment the understanding of linkages between the contaminants concentrations in sediments of the stormwater detention ponds and their toxicity, an integrated approach is essential. In this paper we integrate results of the toxicological and geochemical seasonal survey of the stormwater detention ponds, in order to examine the relation between the chemistry of sediments and their toxic responses.

Study area

Four Metropolitan Toronto stormwater detention ponds receiving runoff from diverse catchments were selected for the study. Col. S. Smith Pond received runoff from a commercial/industrial catchment, while the Heritage Estates and Unionville Reservoirs received runoff from residential areas. One pond, Tapscott Reservoir, was located in an undeveloped catchment. A more detailed description of the study area is provided in Mayer et al. (1996).

FIELD PROCEDURES AND METHODS

Sample collection

Suspended and bottom sediments were collected from all investigated ponds at different times of the year. The sampling dates were selected to reflect characteristic seasonal variances. To assess the ambient conditions in the ponds at the time of sampling, field measurements of temperature, turbidity, pH, conductivity and dissolved oxygen were carried out and water samples were collected for metals and nutrients analyses. Suspended sediments were collected using a Westfalia centrifuge system and the bottom sediments were collected using an Eckman dredge. The sample collection techniques were the same for all reservoirs. Sampling was carried out near inlet, outlet, and if the conditions permitted, in the middle of individual reservoirs. Further details of the sampling procedures are provided in Mayer et al. (1996).

Bioassays and chemical analyses

The toxicity of sediments was evaluated using the battery of tests approach. This approach utilizes different physiological endpoints and it is recognized that organisms from the same or different trophic levels or occupying different ecological niches respond differently to a multitude of contaminants (Day et al., 1995; Dutka and Kwan, 1982; Dutka et al., 1988).

Bottom and suspended sediments and their extracts were subjected to bioassays previously described by Dutka (1989) and Dutka et al. (1990). Bioassays for bioavailable toxicants and genotoxicants were carried out on pore (interstitial) and extruded water of bottom and suspended sediments, respectively, and on the sediment solvent extracts (10% dimethyl sulfoxide + 10% methanol, Dutka et al., 1994a, 1994b). Direct sediment toxicity tests were also performed, as it is thought that these tests assesss the bioavailable toxicants and have significant ecological relevance when predictions are to be made for organisms living in sediments (Day et al., 1995). The toxicity tests used on the sediment extracts included SOS-Chromotest, Microtox® test and mammalian submitochondrial particles (SMP) test. In addition to these tests, *Daphnia magna* (48 h acute toxicity and 7 d chronic toxicity tests), seed germination and root inhibition (with butter crunch lettuce, *Lactiva sativa*) and the MetPAD tests were performed on sediment pore/extruded water. A description of these bioasssays can be found elsewhere (Dutka et al. 1994a, 1994b, 1996). The direct sediment toxicity tests included Direct Sediment Toxicity Testing Procedure (DSSTP), also known as Sediment Chromotest® and the Microtox® Solid Phase Test (SPT). A brief description of the tests follows.

SOS-Chromotest is a test for the presence of bioavailable genotoxicants which is based on a colorimetric assay of microbial enzymatic activities (β-galactosidase activity or SOS response activity) after incubating the tester strain (*Eschericia coli* K12-PQ37) with a suspected liquid sample. The test was performed with and without the addition of S9 (Arochlor induced rat liver homogenate). Response was considered positive, when induction factor was >1.3.

Microtox® test, developed by Microbics Corporation is a photometric technique, that measures inhibition of bioluminescence in a marine bacterium *Vibrio fischeri*. The endpoint of this toxicity assay is 50% reduction of the light emission intensity and the data are reported as EC_{50} (% of sample producing 50% light inhibition by the test bacteria).

The two SMP systems tests, which use beef heart submitochondrial particles to screen for for bioavailable toxicants, were forward electron transport (FET) and reverse electron transport (RET). The FET is based on the forward movement of electrons from the NADH through mitochondrial enzyme complexes I, III, and IV converting it to NAD. In the RET system the energy supplied by the ATP drives the electrons in the thermodynamically unfavorable direction through mitochondrial respiratory enzyme complex I from NAD, converting it to NADH. The conversion in both cases is monitored spectrophotometrically. The results are reported as % of reaction inhibition compared to a non-toxic control. Inhibition between 30-50% is considered suggestive of toxicant presence, while inhibition greater than 50% is taken to be confirmation of toxicity.

DSTTP commercially sold as Sediment Chromotest is adapted (Kwan and Dutka 1992a, 1992b) for environmental solid phase samples from the liquid phase test known as the Toxi-Chromotest (Organics Ltd. 1985). The assay is based on the ability of toxicants to inhibit *de nova* synthesis of the inducible enzyme, β-galactosidase, in an *E. coli* strain which is highly sensitive to a broad variety of chemicals. The activity of the enzyme is detected by reacting with a chromogenic substrate that can be measured visually or colorimetrically. Data are expressed as sample concentrations (% of sample) producing 100% inhibition of the β-galactosidase production (EC_{100}).

Microtox® solid-phase test (SPT) measured inhibition of bioluminesce in *V. Fischeri* by directly exposing the bacterium to an aqueous suspension of sediment. This suspension is then tested for bioluminescence using procedures similar to those in the routine Microtox® test and the results are reported as the sample concentrations (%) required to produce an EC_{50} effect. Sample concentrations greater than 2% are regarded as negative , and samples between 1 and 2% are doubtful or suggestive. A more detailed description of the assays is given in Dutka et al. (1996). Suspended and bottom sediments were analyzed for the total concentrations of heavy metals, total and organic carbon concentrations and phosphorus. The details of all the analytical procedures used are given in Mayer et al. (1996).

Table 1. Toxicity tests responses and heavy metals concentrations in bottom sediments of the surveyed ponds (p.w. = pore water, s.e. = solvent extract)

Site		Sampling Date	Sample I.D.	DSTTP % Sed. to EC100	SPT Produce EC 50	SOS Chromotest Induction Factor (p.w.) -S9	SOS Chromotest Induction Factor (p.w.) +S9	SOS Chromotest Induction Factor (s.e.) -S9	SOS Chromotest Induction Factor (s.e.) +S9	Microtox % s.e. EC 50	Cl mg/L	TP mg/L	NH3 mg/L	Fe mg/g	Mn mg/kg	Zn mg/kg	Pb mg/kg	Cu mg/kg	Cd mg/kg	Ni mg/kg	Cr mg/kg
Heritage	Inlet	92/11/11	S45	2.5	nt					26.7	21.6	0.0667	0.181	23.7	494	129	38.4	30.8	1.76	28.6	23.0
			S44	2.50										17.8	442	145	47.2	30.4	1.62	24.2	19.6
	Middle		S43	2.50	nt				1.6	13.5	22	0.0657	0.167	15.2	354	109	34.0	24.0	1.34	19.4	17.4
			S42	2.50										24.1	525	173	50.6	34.8	2.98	29.8	23.6
	Outlet		S41	2.50	nt		1.6				21.5	0.0737	0.178	25.2	540	172	50.6	36.2	4.74	26.4	28.0
Heritage	Inlet	93/05/04	Inlet	8.30	0.35		1.4	1.6	1.3	9.7	238	0.0318	0.146	14.0	344	124	34.0	23.6	1.66	16.6	13.8
	Middle		Middle	2.10	0.19	1.4	1.6			9.5	239	0.0409	0.159	23.6	530	160	47.2	32.6	2.42	26.2	25.6
	Outlet		Outlet	16.70	nt	1.3	1.6		1.3	5.9	241	0.0354	0.143	26.5	524	141	46.0	31.2	2.02	25.0	24.0
Heritage	Inlet	93/08/19	Inlet	1.10	0.15				1.8		49.4	0.0607	0.32	22.5	528	145	57.0	33.2	2.12	32.0	31.4
	Middle		Middle	33.30	0.17						48.8	0.0755	0.384	22.3	547	143	51.4	34.0	1.92	31.0	28.8
	Outlet		Outlet	33.30	0.31						49.9	0.0471	0.234	19.7	464	167	53.2	38.6	2.30	29.6	29.8
S. Smith	Inlet	92/10/14	S5								135	0.1666	0.174	26.6	677	691	209.6	163.6	4.76	50.8	44.8
			S4											25.2	701	659	210.8	162.8	4.12	54.2	42.4
	Middle		S3			1.5					192	0.1066	0.206	23.6	618	513	228.2	142.0	3.76	44.4	37.2
			S2											25.6	904	726	256.4	156.6	3.70	39.2	59.6
	Outlet		S1								175	0.1158	0.005	25.6	684	509	156.4	125.0	3.88	44.2	41.0
S. Smith	Inlet	93/05/07	Inlet	16.70	0.25	1.4	1.3	1.3		9.2	167	0.102	0.326	26.1	754	761	230.0	180.0	5.14	55.0	47.6
	Middle		Middle	8.30	0.25		1.3	1.4	1.3	13.2	177	0.0982	0.307	22.1	557	316	95.0	62.4	2.28	28.8	22.4
	Outlet		Outlet	8.30	0.26		1.4	1.3		3.5	170	0.103	0.322	21.9	673	602	196.7	130.0	4.12	40.4	38.8
S. Smith	Inlet	93/08/16	Inlet	33.30	0.35		1.3				36.8	0.1319	0.319	23.0	810	792	241.0	206.6	5.50	50.8	64.4
	Middle		Middle	16.70	0.35		1.4		2.1		39.2	0.1323	0.33	20.7	658	646	211.4	181.0	4.65	48.9	51.4
	Outlet		Outlet	33.30	0.13						40.1	0.1275	0.017	19.3	586	492	186.6	153.6	3.84	42.4	48.6
Tapscott	Inlet	92/10/27	S25	5.00										17.0	341	162	36.2	22.0	1.60	19.4	16.4
			S24	2.50										26.4	495	168	46.0	33.8	2.10	25.6	22.6
	Middle		S23	2.50										26.1	478	168	47.2	33.6	1.96	30.8	22.2
			S22	1.25										30.6	524	208	52.8	40.0	2.16	33.0	30.2
	Outlet		S21	2.50										30.3	557	197	50.6	36.6	2.10	33.6	28.0
Tapscott	Inlet	93/05/06	Inlet	16.70	0.84		1.6	1.4	1.4	22.7	105	0.0731	0.111	23.6	457	150	42.0	29.0	2.20	31.4	20.4
	Middle		Middle	8.30	0.54	1.4	1.4	1.3	1.3	13.4	105	0.0491	0.089	26.5	501	180	43.2	32.2	1.96	32.6	22.8
	Outlet		Outlet	33.30	0.10		1.4	1.4	1.3	11.3	103	0.0554	0.071	27.7	541	188	48.8	36.2	2.20	30.6	23.6
Tapscott	Inlet	93/08/18	Inlet	33.30	0.26						58.7	0.0525	0.16	20.3	471	149	43.6	31.8	2.10	28.8	21.0
	Middle		Middle	33.30	0.25						58.3	0.1441	0.244	21.8	485	197	48.2	37.2	1.86	31.8	27.4
	Outlet		Outlet	8.30	0.19			1.3			59.7	0.1782	0.258	26.8	553	190	50.0	41.2	1.88	33.2	29.4
Unionville	Inlet	92/11/12	S61						1.3	14	28.4	0.136	0.262	5.4	144	40	22.8	7.0	1.28	5.8	7.2
			S62	5.00	0.40									19.6	411	105	48.4	29.6	1.80	24.2	21.8
	Middle		S63	5.00	0.40		1.6		1.7	5.4	71.8	0.0913	0.403	24.2	551	109	52.8	31.8	2.18	25.0	23.0
			S64	2.50										23.1	497	127	57.2	36.6	2.42	27.2	22.2
	Outlet		S65	5.00			1.4		1.6	5.2	59.5	0.1434	0.324	22.3	448	120	59.4	35.4	2.30	26.4	20.8
Unionville	Inlet	93/05/05	Inlet	33.30	1.40		1.3				280	0.0822	0.234	6.5	133	32	26.2	13.6	1.16	8.4	10.2
	Middle		Middle	16.70	0.12						280	0.0935	0.269	25.2	470	114	53.8	35.4	2.08	24.8	26.2
	Outlet		Outlet	8.30	0.16						279	0.0746	0.233	21.1	420	117	60.4	38.6	2.32	21.0	20.4
Unionville	Inlet	93/08/17	Inlet				1.3			17.2	98.2	0.089	0.179	7.0	132	40	25.6	12.8	1.12	12.4	8.4
	Middle		Middle	33.30	0.16	1.3	1.3		1.3	13.3	98.8	0.0885	0.396	21.3	485	128	58.0	41.6	2.50	38.8	36.8
	Outlet		Outlet	33.30	0.08	1.3	1.3	1.3		13.9	101	0.0526	0.178	17.5	455	146	63.6	52.8	2.84	36.2	35.8

RESULTS

The majority of the toxicity data were obtained from microbial bioassays. Concerns have been raised about the ecological relevance of bacterial bioassay as they are unrepresentative of freshwater ecosystems (Blum and Speece 1990; Day et al., 1995; Chapman et al., 1996). However, these assays are rapid, inexpensive and they produce reproducible results which can be compared and correlated with the toxicity tests utilizing other species.

The toxicity tests indicated a widespread presence of toxicants and genotoxicants. No clear seasonal pattern emerged from the toxicity tests nor from the distribution of heavy metals in suspended and bottom sediments (Table 1). Similar toxic responses were observed for inlet and outlet sediments, suggesting similar levels of toxicants at the inlets and outlets of these ponds. Positive toxic response of sediments is interpreted here as being indicative of the presence of bioavailable toxicants and genotoxicants. Similarly, there were no substantial decreases in heavy metals concentrations in sediments (Table 1) from entrance to exit.

All pore water samples tested negative using the MetPAD test, which is based on the enzyme inhibition of a strain of bacteria by bioavailable heavy metals. Twenty five pore water samples were found to contain substances with genotoxic activity, with 76% of them being promutagens (requiring S9 activation). S. Smith and Unionville ponds ranked the highest (each of them accounting for 8 positive responses). It appears that sediments collected in May had the highest loads of genotoxicants. All pore water samples of Unionville sediments also produced inhibiting effect on the growth of the buttercrunch lettuce root (>25%). Pore waters of S. Smith ranked second in this root inhibition test, while just only few samples from Tapscott and Heritage ponds showed slight toxicity. Only pore water samples of Col. S. Smith sediments collected in May, produced positive Microtox® responses and the values for inlet, (26.6- 38.5% sample producing EC_{50}). The sediments of this reservoir had also the highest heavy metals concentrations (Table 2) and oil and grease content (unpublished data). The pore water data suggest that the two ponds (S. Smith and Unionville) were more impacted by water soluble toxicants than the remaining ponds, which is consistent with the concentrations of heavy metals in the sediments of the investigated ponds. Partitioning of metals between the aqueous and solid phases is redox sensitive. Reducing conditions beneath the sediment-water interface result in increased levels of dissolved metals in pore water. It is, therefore, assumed that metals more than organic contaminants, which are generally less water soluble, are responsible for the toxicity of pore water. Due to the lack of response to *D. magna* acute toxicity test, *D. magna* chronic toxicity test was carried out on sediment pore waters. Only one pore water sample from May sampling of the Heritage pond tested positive and one sample from S. Smith pond tested positive by *Spirillum volutans* test.

Principal component analysis (PCA) was employed to reduce the multidimensionality of the multi variate data set. The results showed that three significant factors (principal components) explain 83% of data variance. The first factor grouped together heavy metals. The second factor which explained 17% of variance, with high loadings of Fe and slightly lower loadings of DSTTP, SPT and SOS (+S9), suggests that increasing Fe concentrations in sediments decrease availability of toxicants and vice versa. Stronger binding of heavy metals to solid phase, due to the strong absorption on Fe-oxides, may be the probable explanation for negative responses of pore water samples in MetPAD bioassay, developed specifically to screen for heavy metals in liquid samples.

Twenty four sediment solvent extracts were found to provide toxic responses in the bioassays, with majority of them occurring in May. Sediments solvent extracts also showed more positive Microtox® responses (Table 1) than did the pore water samples. SMP assays of solvent extracts of sediments also indicate more positive responses than those of pore water. Solvent extracts of S. Smith sediments showed most positive responses. Thus, the toxicity data indicate that the solvent extracts of sediments were more toxic than pore waters, suggesting higher concentrations of organic toxicants/genotoxicants, which require solubilization by organic solvents.

Table 2. Mean heavy metals concentrations in suspended and bottom sediments

Metal	Sed.	Tapscott mg/kg	Heritage mg/kg	S. Smith mg/kg	Unionville mg/kg	LEL[1] mg/kg	SEL[2] mg/kg
Zn	Sus.	244	282	1077	245	120	820
	Bot.	178	146	610	98	120	820
Pb	Sus.	58	61	265	83	31	250
	Bot.	46	46	202	48	31	250
Cu	Susp.	44	59	215	66	16	110
	Bot.	34	32	151	30	16	110
Cd	Susp.	2.11	2.32	6.22	2.98	0.6	10
	Bot.	2.01	2.26	4.16	2.00	0.6	10
Ni	Susp.	34.8	29.8	49.7	31.2	16	75
	Bot.	30.1	26.3	45.2	22.7	16	75
Cr	Susp.	36.1	29.3	55.2	34.5	26	110
	Bot.	24.0	24.1	45.3	21.2	26	110

[1] MOEE Guidelines, LEL = low effect level
[2] MOEE Guidelines, SEL = severe effect level

Large number (20) of water samples extruded from suspended sediments and 17 solvent extracts of suspended sediments tested positive for the presence of the promutagens (SOS-Chromotest +S9) while only two extruded water samples and four solvent extracts tested positive for direct acting genotoxins (SOS-Chromotest -S9). The distribution of water and solvent soluble genotoxins did not differ much between the ponds. Induction Factors ranged between 1.3 and 1.6 (Dutka et al., 1994b). Highest Induction Factors, 3.8 and 2.1, were found for water extruded from suspended sediments of Unionville and Heritage Ponds (Dutka et al., 1994b), respectively, from November sampling, when continuous rain resulted in the scouring of the streets and a significant input of street dust which among other contaminants carried high levels of heavy metals (Mayer et al., 1996). During rainfalls, water rushes along pavement and yards, accumulating untreated pollutants such as metals, pesticides and oil. The storm drains collect this runoff and ultimately transport it, untreated, into the stormwater detention ponds, raising toxicant levels in the ponds.

The toxicity data (Table 1, Dutka et al., 1994b) suggest that suspended sediments contain higher water soluble toxicant loads than bottom sediments, what is consistent with the concentrations of metals in suspended and bottom sediments. As seen from Table 2, the mean metal concentrations of suspended sediments are higher than those of corresponding bottom sediments.

SUMMARY

This study presents an overview of the seasonal survey of urban stormwater detention ponds. Toxicological data were related to sediment chemistry and ambient conditions in the ponds to determine the extent and environmental significance of sediment contamination. A variety of bioassays, using different endpoints, were employed to assess the toxicity of bottom and suspended sediments. The data reveal a widespread presence of toxicants and genotoxicants in suspended and bottom sediments

in all investigated ponds. No obvious decrease in toxicity or heavy metals concentrations from entrance to exit was observed, suggesting that under the conditions studied the ponds have only limited ability to remove sediment-associated contaminants. The survey showed elevated levels of heavy metals in ponds receiving runoff from industrial/commercial and residential areas. These ponds also ranked higher in the toxicity tests.

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THE CHARACTERIZATION AND TOXICITY TESTING OF THE BASE/NEUTRAL FRACTION FROM POREWATER AND SOLIDS FROM OIL SANDS FINE TAILINGS

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In the Athabasca river basin, an average of 600,000 tonnes of oil sands/day are processed by the Clark caustic hot water process, extracting bitumen and releasing large volumes of tailings which separate into sand and mature fine tails (MFT). A 'zero discharge' policy for potentially toxic MFT results in a growing storage problem. One method of MFT reclamation currently undergoing environmental risk assessment is the wet landscape strategy. In the present project, the base/neutral (B/N) fraction of the MFT will be separated, characterized for organic compounds, and tested for toxicological properties.

The MFT were divided into pore water (PW) and fine tailings solids (FTS) by centrifugation. Separation of the dichloromethane extract of FTS by pH and *n*-pentane solubility produced the maltene fraction. Primary separation of the maltene fraction by silica generated four fractions: the first contained saturated compounds; the second contained aromatic compounds, noted for their mutagenic and carcinogenic potential. Preliminary analysis by GC/MS has revealed the presence of alklyated and non-alklyated polycyclic aromatic compounds (PACs).

Mutatox®, is a simple, rapid and sensitive screening tool that can detect potential genotoxic substances in complex environmental mixtures by measuring the light output from a dark mutant strain (M169) of the luminescent bacteria, *Vibrio fischeri*. Preliminary work on the B/N fractions has shown that mutagenic potential exists in the PW B/N fraction and FTS aromatic fractions. As Mutatox® is still in the validation stages, it will be compared to the well documented and established Ames *Salmonella* Assay for PACs.

TIE STUDIES ON WATERS AND WASTEWATERS OF A PROPANE PLANT IN ALBERTA

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This study reports the TIE investigations carried out on waters and wastewaters of a gas processing plant in Alberta, Canada. The studies were conducted to determine the role of a biocide (used as anti-fouling agent), chlorine, and a corrosion inhibitor, in the toxicity of the plant's waters and wastewaters. The results showed that consecutive biocide treatments produced persistent toxicity; the corrosion inhibitor did not seem to contribute to the toxicity; and removal of chlorine and the non-polar compounds seem to detoxify the test samples.

TIE OF EFFLUENT FROM A CHEMICAL MECHANICAL THERMAL PULP MILL: A TREATABILITY APPROACH

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In late 1995 a final effluent sampled from Millar Western Pulp Mill failed the trout test with an eventual 96-h LC_{50} of 33%. The trout exhibited haemorrhaging under and around the gills, pale colouration, and some dermal peeling. The pH was subsequently manipulated in the *Daphnia* test and the toxicity of the effluent dramatically increased as the pH decreased. These observations were consistent with organic acid toxicity. Millar Western was contacted and they immediately initiated a TRE in which they took several mitigative actions, to reduce resin acid levels. These actions included the following [1] addition of coagulant to facilitate solids removal, [2] reseeding of the basin with healthy activated sludge, and [3] increasing the addition of ammonia to enhance biological activity. Analysis of resin and fatty acids with GC/MSD, showed that the resin acid component of the effluent, in particular dehydroabietic acid, was extremely elevated compared to historical levels. Millar Western immediately ceased discharge to the river and reduced pulp production. Literature values state the LC_{50} value of dehydroabietic acid

alone range between 1.03 mg/L and 1.74 mg/L. Our results showed an LC_{50} value of approximately 2.5 mg/L of dehydroabietic acid in the whole effluent if one assumed no antagonisms. As the resin acid and dehydroabietic acid concentrations declined due to the improved treatment, the trout and *Daphnia* toxicity declined to non-toxic conditions and flow to the river was resumed. The mill's effluent matrix is now similar to that before the time of failure and no toxicity has been observed since.

INCORPORATION OF TOXICITY TESTS AND SPMDS IN THE DESIGN OF ENVIRONMENTAL IMPACT ASSESSMENTS

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Environmental Impact Assessments (EIAs) are performed to support new industrial initiatives and site decommissioning and cleanup. The most common method employed is to incorporate industry information, supplemented by chemical analyses of process and receiving environment samples, into a human and/or ecological risk assessment. The chemical information most often requested is for the analyses of priority pollutants and GC/MS scans. The problem with such an approach is that priority pollutant and GC/MS analyses represent a small portion of known chemicals. Furthermore, toxicological information is largely lacking for specific biological endpoints, hence, rigorous use of structure activity relationships must often be incorporated in human and/or ecological risk assessments.

A study was conducted in which a battery of aquatic toxicity tests (acute and chronic) endpoints) were used to assess aquatic protection issues. Semi-permeable membrane devices (SPMDs) were included to simulate bioconcentration processes. The SPMD dialysates were subjected to an innovative suite of toxicity tests (Microtox®, SOS-Chromotest, and Mutatox®) and chemical analyses to assess potential human health effects from bioconcentratable toxic substances. The approach is novel to EIA work in that toxicological rather than chemical specific endpoints are incorporated and hence a better assessment of environmental and/or human health protection can be made.

BINDING OF INORGANIC MERCURY TO GILLS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Rainbow trout (*Oncorhynchus mykiss*, 5-10 g) were exposed to ~1.0 mM of inorganic mercury ($HgCl_2$) in synthetic soft water (Ca, Na ~300 mM, pH 6.5-7.5). To the water synthetic ligands (ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), cysteine, ethylenediamine, or dissolved organic carbon (DOC)) and cations (Ca, Na, or H^+) were added. After 2 to 3 h the fish were removed from solution, the gills extracted, and the amount of Hg bound to the gills was examined using Cold Vapour Atomic Absorption Spectroscopy (CV-AAS). Using these results, a calculation of the conditional equilibrium binding constant (K) at the gills was made (log $K_{Hg\text{-}gill}$ ~17.5), with ~30 nmol of Hg binding sites per gram of gill tissue. Stability constants for DOC, Ca, Na, and H^+ with the gills were estimated and inserted into the MINEQL$^+$ aquatic chemistry program. This modification of the MINEQL$^+$ program will allow the prediction of Hg accumulation by the gills when fish are exposed to inorganic Hg in the aquatic environment.

EFFECTS OF TEMPERATURE AND THIOSULPHATE ON UPTAKE OF SILVER BY RAINBOW TROUT (*Oncorhynchus mykiss*)

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We are using silver as a 'model' metal to better understand the effects of temperature and complexing agents such as thiosulphate on metal uptake by fish. We have exposed rainbow trout (*Oncorhynchus mykiss*, ~210 g) to 0.1-0.2 mM Ag (as $AgNO_3$) in soft water for five days at 12°C and 21°C, and in the presence or absence of 5.0 mM S_2O_3 (as $Na_2S_2O_3 \times H_2O$). The trout are cannulated *via* the dorsal aorta to allow repetitive blood sampling. We monitor blood PO_2, PCO_2, pH, and lactate, plus plasma Na, Cl, Ca, glucose, ammonia, and Ag. Plasma Ag indicates the rate of active and passive Ag entry across the gills and into the blood.

Our results to date indicate that Ag accumulation in trout plasma is an active uptake process with plasma Ag concentration increasing from 0.05 mM to 1.6 mM over a 5 day exposure to 0.1-0.2 mM Ag. However, our data do not yield a difference in plasma Ag concentration between the cool and warm groups of fish. That is, we can not speed up active Ag uptake by increasing water temperature to 21°C. We are currently trying to slow down active Ag uptake by exposing adult trout to Ag at colder temperatures (~5°C) and 18°C to determine if depressed metabolic rate has an effect on active metal uptake by trout.

ESTROGEN STIMULATES GROWTH HORMONE AND GONADOTROPIN SUBUNIT GENE EXPRESSION IN THE GOLDFISH PITUITARY

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The effect of a natural estrogen (17β-estradiol) on pituitary Growth Hormone (GH) and Gonadotropin (GtH) subunit gene expression was investigated *in vivo* and *in vitro*, using goldfish as an experimental model. Early to mid-recrudescent goldfish were anaesthetized and injected intraperitoneally with 20, 200, and 2000 ng of 17β-estradiol. Fish were sacrificed after 24 hours and pituitaries were removed and used to extract ribonucleic acids (RNA). Total RNA was separated on a 1.2% formaldehyde agarose gel and transferred to a hybond N+ membrane using the capillary transfer method for Northern analysis. A stimulation of the GH messenger RNA (mRNA) level was observed at all concentrations used, with a greatest stimulation occurring at a dose of 20 ng/fish. Injection with 17β-estradiol also stimulate GtH subunit mRNA levels, and the magnitude of the stimulation was found to be greater than that of GH. These results indicate that 17β-estradiol has a stimulatory effect on both GH and GtH subunit mRNA levels, *in vivo*.

To investigate the effect of 17β-estradiol at the level of the pituitary, experiments were performed on cultured goldfish pituitary tissue, *in vitro*. Goldfish pituitaries were removed, fragmented, and superfused with media containing 17β-estradiol at concentrations of 2, 20, and 200 ng/ml. Treatment with 17β-estradiol *in vitro* stimulated both GtH and GH mRNA levels at all concentrations tested. Similar to the *in vivo* studies the magnitude of stimulation was greater for the GtH subunits, compared to that of GH. Overall, these results demonstrate that 17β-estradiol stimulates both GH and GtH subunit mRNA production in the goldfish pituitary. The stimulatory effect of estrogen has been demonstrated to occur directly at the level of the pituitary gland.

ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF NON-PLANAR POLYCHLORINATED BIPHENYLS AND AROCLORS

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In vitro recombinant receptor/reporter gene assays were used to examine the estrogenic and antiestrogenic activity of 9 non-planar polychlorinated biphenyls (PCBs) and 8 Aroclors. Human breast cancer cells (MCF-7) transiently transfected with a Gal4-human estrogen receptor chimeric construct (Gal4-HEGO) and a Gal 4-regulated luciferase reporter gene (17m5-G-luc), and HeLa cells stably transfected with Gal4-HEGO:17m5-G-luc exhibited partial agonist activity (< 40% of 17β-estradiol) following a 24 h exposure to 10 μM of various non-lanar PCB congeners. The order of estrogenic potency of PCBs, ranging from 1000 to 10,000,000 times less potent than 17β-estradiol, were as follows: 2,2',6,6'-tetrachlorobiphenyl (PCB 54) > 2,2',4',,6,6'-pentachlorobiphenyl (PCB 104) > 2,2',4,6-tetrachlorobiphenyl (PCB 50) > 2,6-dichlorobiphenyl (PCB 10) > 2,4,4',6-tetrachlorobiphenyl (PCB 75) > 2,2',6-trichlorobiphenyl (PCB 19) > 2,3,4,5-tetrachlorobiphenyl (PCB 61) > 2,3,4-trichlorobiphenyl (PCB 21) > 2,2',4,4',6,6'-hexachlorobiphenyl (PCB 155). Luciferase activity was abolished upon cotreatment with the pure antiestrogen ICI 164,384. Aroclors 1221, 1232, 1016, 1242, 1248, 1254, 1260, and 1268 were inactive at the highest concentration tested (10 μM). Our preliminary results demonstrate that PCBs are capable of interacting with the human estrogen receptor and provides further impetus to study the structure-activity relationship for this class of xenoestrogens.

SENSORY EVALUATION STUDIES OF FISH EXPOSED TO PULP AND PAPER MILL EFFLUENT AT SIX CANADIAN PULP MILLS

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The federal Environmental Effects Monitoring (EEM) program, a component of the Fisheries Act; Pulp and Paper Effluent Regulations, requires sensory evaluation of tainting at Canadian pulp and paper mills where the results of fish contaminant analyses indicate that there is no potential health hazard and where there have been historical complaints of fish tainting or altered fishing efforts. Six Canadian pulp mills undertook sensory evaluation tests as a requirement of their first cycle of EEM; the studies were conducted at pulp mills in British Columbia (3), Alberta (2) and Nova Scotia (1). The sensory evaluation test methodologies employed by the pulp mills varied considerably between the studies, however there was a degree of similarity in the flavour evaluations and corresponding results at all the mills after substantial process changes and improved effluent treatment. Each of the studies evaluated fish flavour using Triangle Difference Test methodology with one Alberta study also incorporating Difference from Control Testing. Results from the Triangle Difference Tests indicated a perceptible difference in the flavour of fish exposed to various concentrations of pulp mill effluent in five of the six studies. The study in Alberta that also evaluated flavour using a Difference from Control Test did not find a significant difference in the flavour between wild fish exposed to effluent and control samples, however a significant difference was found between fish exposed to a 3% concentration of effluent under laboratory conditions and control samples. Four of the taste evaluation studies went one step further and evaluated panelists' preference of fish samples using a Hedonic Preference Test methodology. In each of the four studies, it was found that while there was a perceivable difference in the flavour of fish (triangle difference testing) exposed to pulp mill effluent, when compared to non-exposed fish, there was no difference found as to the preference in the flavour between the samples.

SENSITIVITY OF THE AMPHIPOD *Eohaustorius estuarius* AND THE POLYCHAETE *Neanthes arenoceodentata* TO AMMONIA, A POTENTIALLY TOXIC COMPONENT OF SEDIMENTS

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INTRODUCTION

Ammonia is a common constituent of sediments that may be present at toxic concentrations (USEPA/USACOE, 1993; Kohn et al., 1994). Ammonia toxicity may confound results by obscuring the effects of other contaminants. Alternatively, ammonia toxicity may result in a sediment being classified as hazardous when, in fact, ammonia effects are transitory and not cumulative. This study was conducted to determine the ammonia concentrations associated with toxic effects on two commonly used marine sediment bioassay organisms, the amphipod *Eohaustorius estuarius* and the polychaete *Neanthes arenoceodentata*. These data can then be used to help interpret test results and also to determine whether samples should be purged of ammonia prior to testing. The specific objectives of the study included [1] develop a database on ammonia toxicity to the amphipod *E. estuarius* and the polychaete *N. arenoceodentata*, [2] compare results for water-only and sediment porewater exposures, [3] compare results on basis of short- (96-h) and long-term (10 and 20 d) exposures, [4] compare results on basis of total and unionized ammonia, and [5] determine concentrations that produce no-observable effects.

METHODS AND MATERIALS

The test procedures were based on PSEP (1995). The sediment was obtained from West Beach, Whidbey Island, WA, and the seawater from Burrard Inlet, BC. The amphipods were collected from Beaver Creek, OR (Northwestern Aquatic Sciences), and polychaetes obtained from cultures in CA (Don Reish, California State University, Long Beach). The tests were conducted in 1-L wide mouth glass jars containing 800 mL of test solution. For the porewater exposures, 175 mL sediment was added to each jar. The porewater exposures incorporated five replicates per concentration and two replicates were used for the water-only exposures. The water only exposures were static. The spiked-sediment exposures were static for the amphipods and were renewed three times per week for the *Neanthes* (PSEP 1995). During the sediment exposures, Neanthes were fed a slurry of TetraMin and the amphipods were not fed. Survival was determined for the amphipods and survival and growth for *Neanthes*. The exposure conditions included temperatures of 15±1°C and 20±1°C for amphipods and polychaetes respectively, and dissolved oxygen of ≥60% saturation, salinity of 28±2 ppt, and lightening was continuous for both species. Organisms per replicates were 20 for amphipods and 5 for polychaetes.

Ammonia solutions were prepared from a stock solution prepared from reagent-grade NH_4Cl (Baker) and used to spike the seawater and sediments. The nominal test concentrations of NH_3 were 0, 31.2, 62.5, 125, 250 and 500 mg/L for amphipods, and 0, 25, 50, 100, 200 and 400 mg/L for polychaetes. Even distribution of the ammonia into the porewater was accomplished by thoroughly mixing the sediment and ammonia solution by stirring for 30 seconds. The suspension was then allowed to settle prior to adding organisms. Ammonia concentrations in both the overlying- and porewater were measured with the salicylate method (Hach Chemical Co., 1992). Ammonia measurements were taken on Day 0, 5 and 10 for amphipods and Day 0, 10 and 20 for polychaetes. Total ammonia was converted to unionized ammonia on the basis of temperature, pH and salinity.

LC_{50}s and no-observable effect concentrations (NOECs) were calculated based on total and unionized ammonia concentrations. LC_{50} estimates were obtained using trimmed Spearman-Karber (Hamilton et al., 1977) and NOEC estimates were determined by Dunnett's test in the analysis of variance (Steel and Torrie, 1960). Data analysis was conducted with TOXCALC (Tidepool Scientific Software, 1994).

RESULTS

Eohaustorius estuarius

The results for the 96 h exposures of *E. estuarius* to ammonia in water are summarized in Table 1. Based on the results of ten studies, the LC_{50}s averaged 143.5 mg/L (total ammonia-N). When analyzed as a function of unionized ammonia, the LC_{50}s averaged 0.8 mg/L (as N). The NOECs averaged 66.5 and 0.5 mg/L (as N) for the total and unionized fractions, respectively.

Table 1. LC_{50} and NOECs for *E. estuarius* exposed to ammonia for 96 h. Values presented at mg/L-N.

	LC_{50}		NOEC	
	Total Ammonia	Unionized Ammonia	Total Ammonia	Unionized Ammonia
Mean±S.D.	143.5±27.8	0.8±0.2	66.5±27.3	0.5±0.2
Min-Max	98.8-191.3	0.5-1.2	31.5-104.0	0.2-1.0
Normal	10	10	10	10

The results from the 10 d spiked sediment studies conducted with *E. estuarius* are presented in Table 2. The LC_{50}s averaged 174.6 and 0.9 mg/L (as N) for the total and unionized fractions, respectively. The NOECs for survival were 95.0 and 0.6 mg/L (as N).

Table 2. LC_{50} and NOECs for *E. estuarius* exposed for 10 d to sediment spiked with ammonia. All values are presented at mg/L-N, as determined in the porewater.

	LC_{50}		NOEC	
	Total Ammonia	Unionized Ammonia	Total Ammonia	Unionized Ammonia
Mean±S.D.	174.6±24.6	0.9±0.1	95.0±24.8	0.6±0.2
Min-Max	155.9-227.1	0.7-1.1	76.4-160.0	0.4-0.9
Normal	9	7	9	7

Neanthes arenaceodenta

Only one 96 h water-only test was conducted with N. arenaceodenta. The LC_{50} estimates, based on the total and unionized fractions, were 115.7 and 1.8 mg/L (as N), respectively. The NOECs for survival were 79.9 and 1.3 mg/L (as N), respectively.

Table 3. LC_{50} values and NOECs for *N. arenaceodentata* exposed to sediment spiked with ammonia for 20 d. All values are presented at mg/L-N, as determined in the porewater on Day 0.

	LC_{50}		NOEC	
	Total Ammonia	Unionized Ammonia	Total Ammonia	Unionized Ammonia
Test 1	119.0	0.9	69.1	0.6
Test 2	98.3	0.6	72.0	0.4

After 20 d of exposure to spiked sediments, the LC_{50}s averaged 109.0 and 0.8 mg/L (as N), based on the total and unionized fractions (Table 3). The NOECs averaged 70.5 and 0.5 mg/L (as N) for these two fractions, respectively. Based on dry weight, the IC_{50}s averaged 72.5 and 0.5 mg/L (as N), respectively, for the total and unionized fractions (Table 4). The NOECs for growth averaged 36.0 and 0.2 mg/L (as N) for these two fractions.

Table 4. IC_{50} values and NOECs for dry weight of *N. arenaceodentata* exposed to sediment spiked with ammonia for 20 d. All values are presented at mg/L-N, as determined in the porewater on Day 0.

	LC_{50}		NOEC	
	Total Ammonia	Unionized Ammonia	Total Ammonia	Unionized Ammonia
Test 1	59.5	0.5	35.6	0.3
Test 2	85.9	0.5	37.0	0.2

Water quality and control performance were within acceptability criteria for all of the tests conducted. Reference toxicant tests ($CdCl_2$) were also conducted with each batch of test organisms. All values were within control limits of the laboratory mean.

CONCLUSIONS AND DISCUSSION

For *E. estuarius*, LC_{50} values ranged between 156 and 227 mg/L in porewater, based on total ammonia (-N). Based on unionized ammonia (-N), the LC_{50} values ranged between 0.7 and 1.1 mg/L. In terms of total ammonia (-N), NOECs for survival in porewater ranged between 76 and 160 mg/L, compared with NOECs for unionized ammonia (-N), which ranged between 0.4 and 0.9 mg/L. Based on unionized ammonia, there was very good agreement in the LC_{50}s and NOECs obtained from the 96-h water-only and 10-d sediment toxicity tests. For this species, unionized ammonia concentrations > 0.2 mg/L-N could result in adverse effects on survival.

For *N. arenaceodentata*, LC_{50} values were 98 and 119 mg/L in porewater, based on total ammonia (-N). IC_{50}s for growth, were 60 and 86 mg/L total ammonia. When calculated as unionized ammonia (-N), the LC_{50} values were 0.6 and 0.9 mg/L in porewater. The IC_{50}s for growth, were both 0.5 mg/L. Based on total ammonia (-N), NOECs for survival were 69 and 72 mg/L in porewater, whereas, the NOECs for growth, were 36 and 37 mg/L. When calculated as unionized ammonia (-N), the NOECs for survival were 0.4 and 0.6 mg/L in porewater, compared with those for growth, which were 0.2 and 0.3 mg/L. In both experiments, growth was a more sensitive endpoint than survival. Based on limited data, the 20 d responses in porewater were approximately half of those observed in the 96-h water-only exposure. These data suggest that, for *N. arenaceodentata*, unionized ammonia concentrations exceeding 0.4 mg/L-N could result in adverse effects on survival and concentrations exceeding 0.2 mg/L could reduce growth.

ACKNOWLEDGEMENTS

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METAL TOXICITY COMPARISON OF A NEW *Hydra attenuata* ASSAY WITH A SUITE OF OTHER TRADITIONAL ACUTE BIOASSAY

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Heavy metal toxicity was assessed with a new bioassay procedure developed with a freshwater cnidarian *Hydra attenuata*. Lethal endpoints (LC_{50}-96 h) obtained with the Hydra test were compared with LC_{50}'s or IC_{50}'s found in the literature for *Escherichia coli, Vibrio fischeri, Selenastrum capricornutum, Daphnia magna* and *Oncorhynchus mykiss*. The Hydra test was found to be the most sensitive assay for detecting the toxicity of chromium. Pb, Zn and Cd showed similar endpoint values for *H. attenuata, D magna* and *O. mykiss*. Comparisons of Hydra LC_{50} values with those of bacteria and algae showed more or less sensitivity according to the substance tested. This preliminary investigation with this simple and cost-effective microassay demonstrates its usefulness in the detection of metal toxicity. Further comparisons with additional chemicals and samples (effluents, pore water) should indicate whether the Hydra assay may prove to be useful within a battery approach for toxicological evaluations.

THE EFFECT OF CREOSOTE ON THE GROWTH AND MEMBRANE INTEGRITY OF THE AQUATIC MACROPHYTE, *Myriophyllum spicatum* L.

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A study has been initiated at the University of Guelph to determine the effects of the wood preservative creosote on the freshwater aquatic environment. Laboratory assays using an aseptic culture of the aquatic macrophyte *Myriophyllum spicatum* L. were conducted to determine the effects of creosote on plant growth and membrane integrity. *Myriophyllum* was exposed to creosote concentrations ranging from 0.16 mg/L to 200 mg/L for 2 weeks. Creosote concentrations up to 12 mg/L stimulated plant growth as measured by shoot length, with higher concentrations inhibiting growth. However, these low concentrations inhibited root growth. From the results of the growth assay, concentrations of creosote corresponding to various amounts of growth stimulation and inhibition were chosen and used to determine the effects of creosote on membrane integrity. Plants were exposed to these concentrations for 4 days, after which membrane integrity was determined by tissue conductivity and potassium leakage. Results suggest that creosote does damage *Myriophyllum* cell membranes, resulting in increased ion leakage. Membrane integrity may be a useful indicator of creosote exposure and effects in the environment.

BENCH SCALE SEDIMENT TREATMENT OF AN INDUSTRIAL SITE

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All treatments effectively stimulated biodegradation of PAHs and oil (TPHs), and decreased concentrations of sulphides. It appears that most of the sulphides and TPHs can be readily biodegraded. Much of the PAH biodegradation in the moderate and high contaminated sediments is probably PAHs dissolved in oil The low contaminated sediment has much less oil and it is likely that in these sediments, most of the PAHs are in pitches no oil. It is highly likely that the PAHs in the oil are more bioavailable than the PAHs in pitch. It is also probable that the PAHs in the pitch are less biodegradable and less bioavailable. Especially in the high contaminated sediments, the production of low molecular weight PAHs from high molecular weight PAHs is obvious. These low molecular weight PAHs readily biodegrade so the process will continue. One hundred and eighty day incubations cannot assess how clean the treated sediments could be, but we are confident that the moderate and high contaminated sediments can be converted to low contaminated sediments. There will likely be a residual of less bioavailable PAHs.

THE USES OF ARTIFICIAL SUBSTRATES AS A MONITORING TOOL

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ABSTRACT

Suspended artificial substrates were used in erosional zones in the Peace River to monitor potential impacts of effluent discharged from Fibreco on the invertebrate community as part of the Environmental Effects Monitoring Program (EEM). Invertebrate communities were dominated by EPT taxa (Ephemeroptera [mayflies], Plecoptera [stoneflies], Trichoptera [caddisflies]), indicating that water quality in the study area was generally good. Oligocheates and chironomids were also observed. EPT abundance was positively related to current velocity, and oligocheate abundance was negatively related to current velocity. These correlations probably reflect the relationship between velocity and abundance of these taxa in natural substrates, as EPT are more lotic and characteristic of erosional areas than are oligocheates. No change in the invertebrate community was observed below the Fibreco outfall. Suspended artificial substrates were generally successful and should be included in the Second Cycle EEM because they identified [1] differences in natural habitat characteristics (i.e., velocity), and [2] differences in invertebrate abundance between some stations and areas.

BACKGROUND

EVS Consultants conducted First Cycle monitoring requirements for Fibreco Pulp (Fibreco) under the Federal Environmental Effects Monitoring (EEM) program. Fibreco operates a Bleached Chemi-Thermo-Mechanical Pulp (BCTMP) mill in Taylor, B.C, which began operation in September 1988. The mill produces approximately 180,000 ADMT (Air Dried Metric Tonne) of pulp per year using a hydrogen peroxide brightening process. The mill does not use chlorine in any of its processes.

The First Cycle EEM Study for Fibreco included the following components: water quality survey, sediment chemistry survey, toxicological testing, and invertebrate community survey for sediments from depositional habitats, invertebrate community survey of erosional habitats using artificial substrates, adult fish survey, and quarterly effluent toxicity testing. The objectives of this study were to evaluate

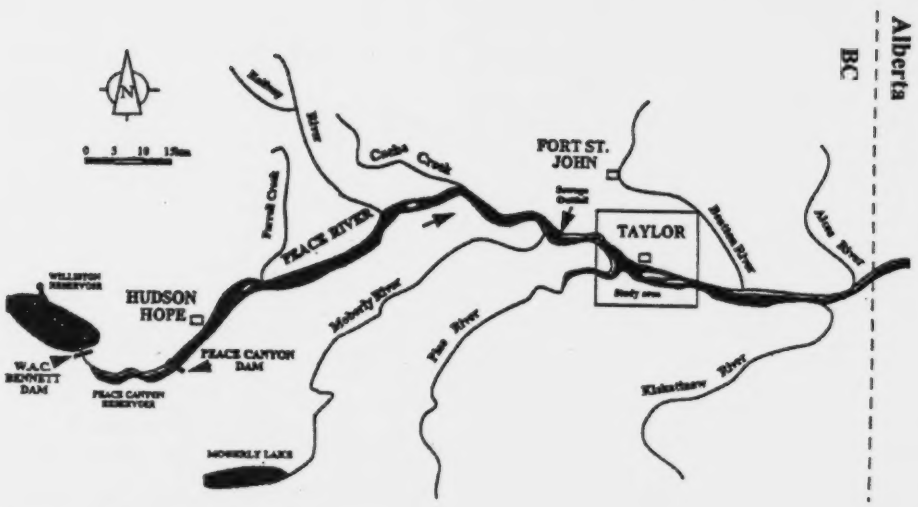

Fig. 1. Map of the Peace River study area.

the use of suspended artificial substrates (SAS) as a tool to conduct invertebrate community surveys to assess the impacts of the Fibreco effluent to the ecology of the Peace River and to confirm the technical feasibility and effectiveness of artificial substrates as principal benthic samplers for erosional habitat surveys for subsequent EEM cycle testing. Results of the other study components will be presented elsewhere.

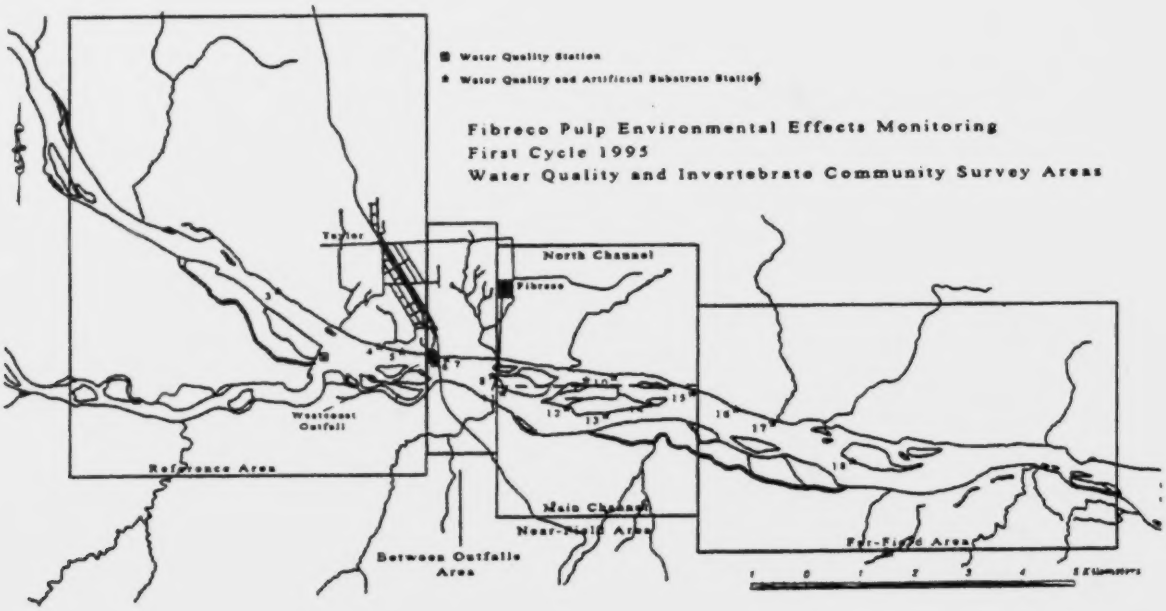

Fig. 2. Overview of invertebrate community survey.

MATERIALS AND METHODS

Study Area and Overall Study Design

Fibreco discharges effluent into the Peace River which flows east from Williston Lake, BC across northern Alberta to join with the Slave River near Lake Athabasca (Fig. 1). The Peace River is British Columbia's second largest river in terms of discharge. Its flow has been regulated since 1967 when the W.A.C. Bennet dam was completed. Flow from tributaries entering between the Pine and the Peace Canyon dams contribute most of the Peace River flow from May to July and September to November. Other effluents (i.e., Westcoast Energy thermal effluent) are discharged into the Peace River approximately 1 km upstream of Fibreco.

Four areas in the Peace River differing in exposure to the Fibreco and Westcoast effluents were surveyed, as part of the First Cycle EEM study (Fig. 2): [1] Reference Station - upstream of the Westcoast outfall; [2] Between Outfalls - between Westcoast and Fibreco outfalls; [3] Near-field - downstream of the Fibreco outfall; and [4] Far-field - downstream of the Fibreco outfall.

Suspended Artificial Substrates

Suspended artificial substrates (SAS) were used to assess the health of benthic invertebrates in the vicinity of the Fibreco mill due to depth and velocity of water in the Peace River, fluctuations in water levels, silty/clay composition of natural shoreline substrate, and safety of the field crew. Comparison of SAS to other methods of artificial substrates is described in Table 1. The SAS consisted of wire barbeque baskets filled with cobble (1.5-3.0 cm diameter: Fig. 3). The SAS were suspended approximately 1 m below the surface from anchored floats (Fig. 4 and 5) at various stations within each of the study areas. Using this method, SAS identify changes and spatial variations in water quality, and not sediment quality because they are not in contact with the sediments, and are colonized mostly by drift organisms.

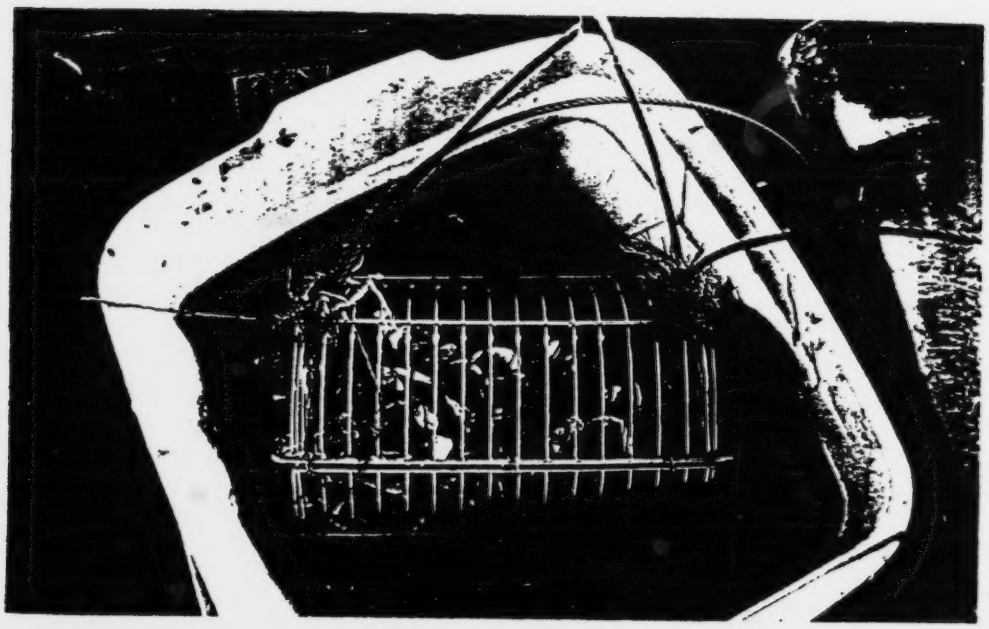

Fig. 3. Photograph of suspended artificial substrates (SAS).

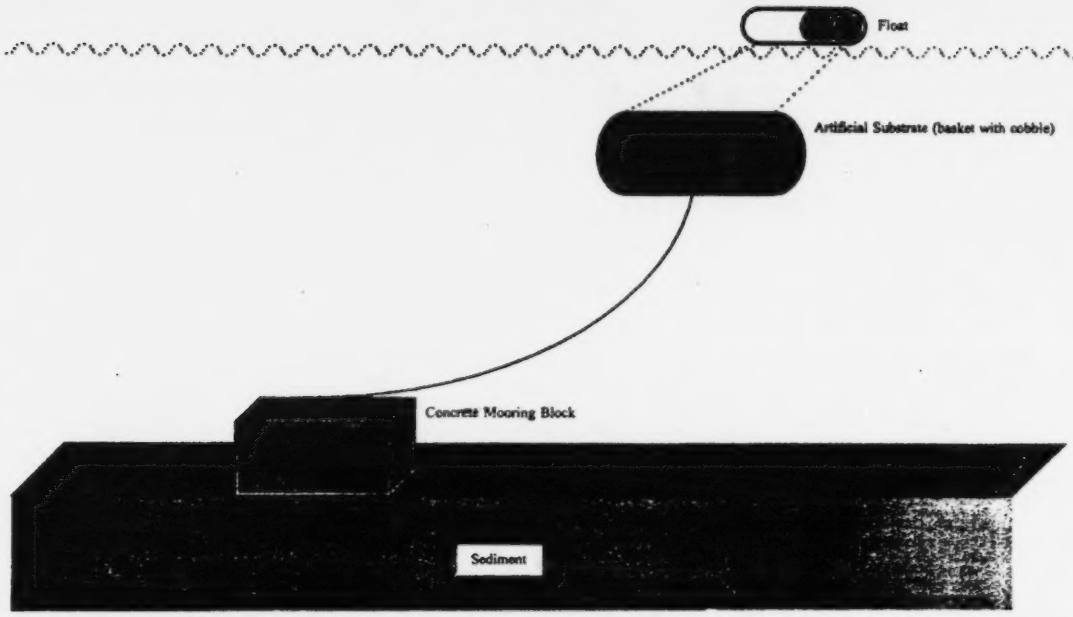

Fig. 4. Schematic diagram of SAS deployed at fixed depth (1 m) below surface and float.

Within each station, the replicate substrates were oriented towards the current and deployed along a transect parallel to shore. The SAS were placed in the river during mid-August and retrieved 6 weeks later in early October, and were checked at least weekly during the six-week exposure to remove accumulation of debris (e.g., branches, leaves, algal mats). Baskets were retrieved using a dip net with 250 um mesh to prevent loss of invertebrates. Invertebrates were then processed for enumeration and identification. Initially, six SAS were deployed at each station to ensure recovery of at least 3 per station. However, a maximum of five SAS per station were processed, with the sixth archived. At one station, fewer SAS were recovered due to vandalism and other factors. The overall approach was to compare communities colonizing SAS among the areas and among stations within areas.

Fig. 5. Photographs of floats in the Peace River.

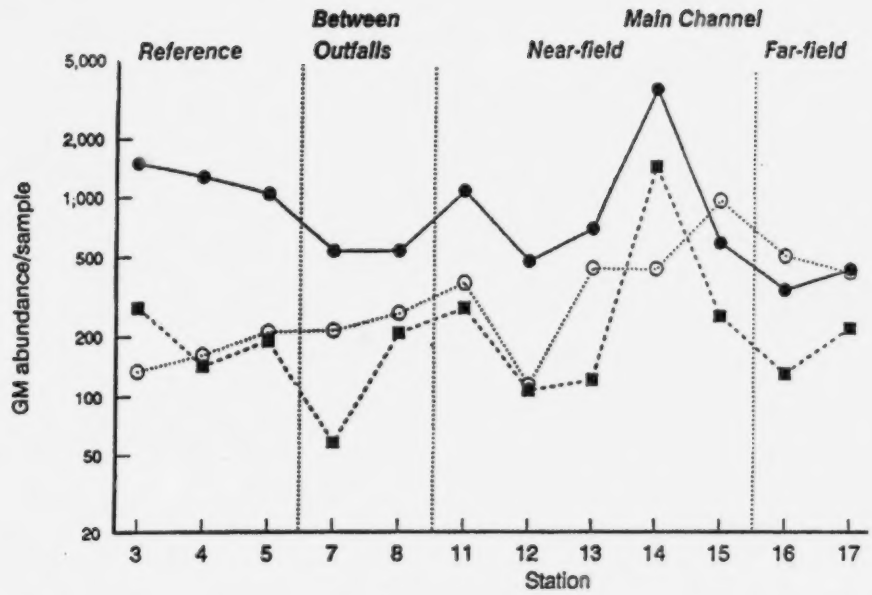

Fig. 6. Spatial trends for major taxon abundances. GM = geometric means.
EPT —●— Chironomidae --■-- Oligochaeta ··⊙···

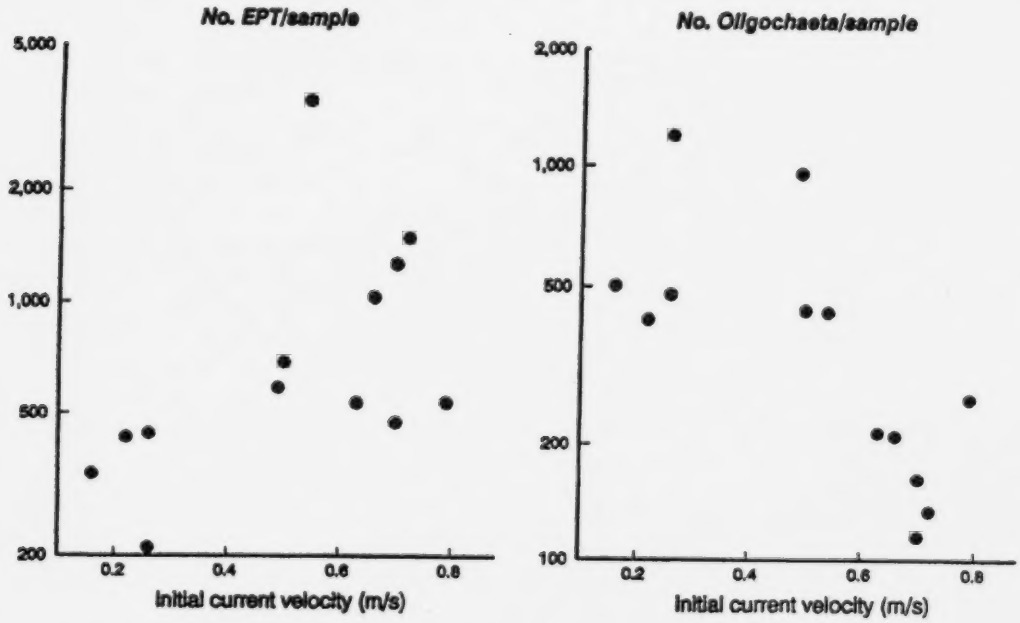

Fig. 7. Relationships between ETP (r_s=0.631, $p<0.05$) and oligochaete (r_s=-0.780, $p<0.01$) abundances (stations GM) and initial velocity.

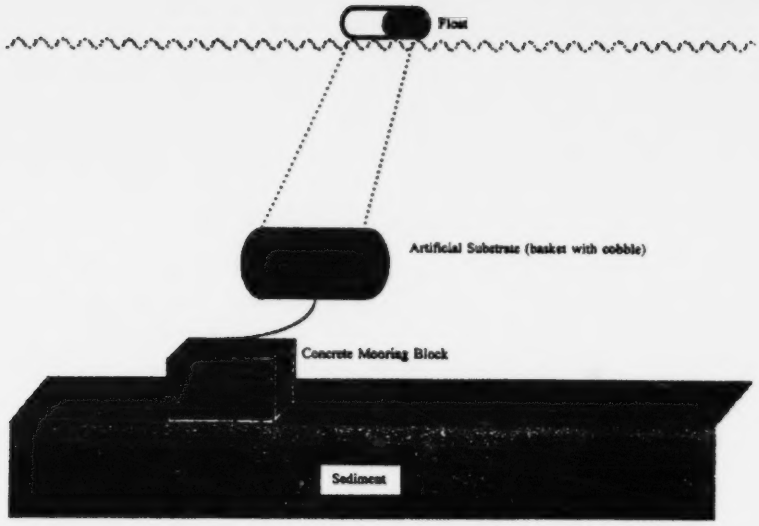

Fig. 8. Schematic diagram of demersal artificial sunbstrate.

RESULTS AND DISCUSSION

The invertebrate communities colonizing the artificial substrates were comprised of 75 different taxa and were dominated by EPT taxa (Ephemeroptera [mayflies], Plecoptera [stoneflies], Trichoptera [caddisflies]). Oligocheates and chironomids were second most abundant taxa. Tubificidae were relatively rare. Dominance of EPT taxa indicate that water quality in the study area was generally good, as these organisms are more sensitive to enrichment and contaminant effects than are other taxa (Klemm et al., 1990). EPT abundance generally decreased and oligocheate abundance generally increased with distance downstream (Fig. 6). EPT abundance was positively correlated (r_s=0.631, $p<0.05$), and oligocheate abundance was negatively correlated (r_s=-0.780, $p<0.01$) with initial current velocity measurements taken in August (Fig. 7). Relationships between biological and physical variables generally indicated strong current velocity effects. These correlations probably reflect the relationship between velocity and abundance of EPT taxa in natural substrates, as EPT are more lotic and characteristic of erosional areas than are oligocheates (Merritt and Cummins, 1978; Brinkhurst and Gelder, 1991). However, as current velocity tended to decrease downstream as the Peace River widens, velocity and potential exposure effects were confounded, but velocity effects were considered real since they were also observed in previous studies (Taylor, 1993). Invertebrate colonization of SAS was mainly by drift (i.e., due to velocity factor) and, thus, may reflect natural communities from upstream areas, not only local communities. Separation of these two sources requires further investigation.

In conclusion, SAS were generally successful because: [1] differences in abundance of invertebrates were observed between some stations and areas; and [2] differences in natural habitat characteristics were identified (i.e., velocity). Based on First Cycle results, the use of artificial substrates as a monitoring tool for invertebrate communities in erosional habitats is recommended for Second Cycle monitoring. However, a number of factors need to be addressed during the Second Cycle so that meaningful data can be collected. The effect of velocity on the invertebrate community needs to be further addressed. This may be done by selecting stations which are consistent in velocity, but this may be confounded by changes in flow regulated by the dam. Alternatively, stations with a wider range of velocities could be selected to test within area velocity effects. In either case, velocity measurements could be taken more frequently.

Table 1. Comparison of different artificial substrates.

Artificial substrate:	
Suspended (SAS)	
Optimal use/habitat:	Deep sites; fast flowing water; large rivers; fluctuations in water levels (e.g., dams); natural silt/clay substrate.
Advantages:	Adequate for erosional habitat; easy recovery of baskets (i.e., no loss of invertebrates; focus on water quality.
Disadvantages:	Accumulation of river debris; focus on water quality instead of sediment quality; focus on drift invertebrates instead of vertical colonization; colonization affected by river current velocity.
Demersal substrates	
Optimal use/habitat:	deep sites; fast flowing water; large rivers.
Advantages:	Adequate for erosional habitat; easy recovery of basket; focus on water and sediment quality; address issues of effects of suspended and deposited solids; simulate local community structure.
Disadvantages:	Colonization affected by river current; difficult deployment and maintenance in large rivers; possible loss of invertebrates upon retrieval.
Sediment trays	
Optimal use/habitat:	Shallow sites; small streams; slow flowing water.
Advantages:	Focus on sediment quality; simulate natural communities; easy deployment.
Disadvantages:	Difficult deployment and recovery in large rivers; possible loss of invertebrates upon retrieval; focus on sediment quality instead of water quality; focus on vertical colonization instead of drift organizations.

If SAS are to be used in future studies, there will be a need to determine the extend of vertical colonization versus drift, to gain a better understanding of the effect of the Fibreco effluent on local communities. This can be addressed by deploying SAS and demersal substrates (see Fig. 8) at the same stations. Invertebrate communities collected below the surface can be compared to communities collected just above the sediment. The use of demersal artificial substrates will also provide a better understanding of the effects of suspended effluent solids close to river bottom.

ACKNOWLEDGEMENTS

We would like to gratefully acknowledge the assistance and cooperation of members of the Taylor Pulp Mill Monitoring Committee, particularly Darren Guliov and Joel Yuen (Fibreco), Al Colodey (Environment Canada), and Dave Sutherland (BC Ministry of Environment, Lands, and Parks). We would also like to thank everyone involved in data collection, data analyses, and report preparation. These include staff members of: EVS Environment Consultants, Environmental Resolution Services, Water Systems Analysts, University of British Columbia, Chemex Labs, Enviro-Test Labs, RL&L Environmental Services, and Len Eklund Jet Boat Services.

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A LABORATORY FORMULATED SEDIMENT INCORPORATING SYNTHETIC ACID VOLATILE SULFIDE

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The usefulness of laboratory formulated sediment (LFS) for sediment research applications might be expanded if sediment characteristics other than particle size distribution, organic carbon and pH could be manipulated. This report describes the development of a LFS containing synthetic acid volatile sulfide (AVS). Several formulations were evaluated with respect to toxicity, chemical stability, and oxidation dynamics. Highest mean survival of *Hyalella azteca* occurred in LFS formulations in which the molar cation-to-sulfide ratio was near 1.0. Suspended synthetic AVS was shown to oxidize within 3 h, but was fairly stable for up to 28 d when isolated from air or oxygenated water. AVS analysis of core slices showed an oxidation profile apparently established and limited by oxygen diffusion. A selected synthetic AVS formulation was evaluated with respect to complexation of Cu, Ni, and Zn with corresponding reduction in metal bioavailability. The toxicity metal-spiked LFS containing synthetic AVS was evaluated by the 10-d *H. azteca* toxicity test. Test responses to amended LFS with metal-to-AVS molar ratios < 1.0 showed some toxicity, apparently due to high iron concentrations. Amended LFS with metal-to-AVS molar ratios > 1.0 significantly affected the responses of both species. The LFS formulation described here may provide consistent and controlled substrates suitable for investigating metal/sediment chemistry and toxicity, and for developing meaningful sediment quality criteria for metals.

A DATA WAREHOUSE FOR TOXIC CONTAMINANTS DATA

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The National Contaminants Information System (NCIS) is a Data Warehouse for managing information on toxic chemicals collected by the Canadian Department of Fisheries and Oceans (DFO). NCIS ensures the long-term security of these toxics data, and provides an intelligent gateway by which to access it. The scope of the NCIS system includes the collection, storage, and provision of access to DFO toxics data. NCIS does not provide analytical (eg. statistics, GIS) capability or reporting functionality. Rather, NCIS can be thought of as a Warehouse where one would go to acquire the 'components' needed for a potentially wide range of possible uses.

The development of NCIS has had to content with a variety of requirements. These ranged from the generally poor state of the departments data holdings, the integration of legacy systems, technical computing issues, and distinctly different information demands by divergent user groups.
In providing a solution to these problems, NCIS was developed as a distributed database system. It allows regions to manage their own data locally, preserves existing archive systems, provides a consistent view of all the department's data holdings, and provides information at a level suitable to distinctly different end user communities.

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BEST STUDENT PAPER AWARDS/PRIX POUR LES MEILLEURS EXPOSÉS PAR DES ÉTUDIANTS

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139	Kierstead	Ted	Nova Chemicals	Box 3081	Sarnia, ON	N7T 8C1	CN
140	Kilgour	Bruce	University of Waterloo	Department of Biology	Waterloo, ON	N2L 3G1	CN
141	Kjersem	Gary	Shell Canada Ltd.	Box 2929 Station M	Calgary, AB	T2P 4V8	CN
142	Klaverkamp	Jack	Freshwater Institute	501 University Crescent	Winnipeg, MB	R3T 2N6	CN
143	Knafla	Tony	Komex International Ltd.	Suite 100, 4500 - 16th Avenue NW	Calgary, AB	T3B 0M6	CN
144	Kovals	Zsolt	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
145	Kreutzweiser	David	Canadian Forest Service	1219 Queen Street East	S. St. Marie, ON	P6H 5M7	CN
146	Kruper	Nancy	Environment Canada	14317 - 128th Avenue	Edmonton, AB	T5L 3H3	CN
147	LaBerge	Don	Chemex Labs Alberta Inc.	2021 - 41 Avenue NE	Calgary, AB	T2E 6P2	CN
148	Labrie	Jacques	ALCAN International Ltd.	1955 Blvd. Mellon P.O. Box 1250	Jonquiere, QC	G7S 4K8	CN
149	LaFleur	Larry	NCASI	P.O. Box 458	Corvallis, OR	97339	USA
150	Lagimodiere	Marie	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
151	Laplante	Dave	Alberta Pacific Forest Industries	Box 8000	Boyle, AB	T0A 0M0	CN
152	Larose	Celine	Concordia University	5860 Waverly	Montreal, QC	H2T 2Y3	CN
153	Latham	Brian	DIAND	P.O. Box 1500	Yellowknife, NT	X1A 2P3	CN
154	Lauten	Karl	Sask. Environ. Resor. Manage.	3211 Albert Street	Regina, SK	S4S 5W6	CN
155	Lawson	Annette	Dundas Environ Consulting Inc.	16 Baldwin Street	Dundas, ON	L9H 1A6	CN
156	Leclair	Gervais	Environment Canada	105, Rue McGill	Montreal, QC	H2Y 2E7	CN
157	Leggett	Sheila	3-D Reclamation Inc.	236, 6715 - 8 Street NE	Calgary, AB	T2E 7H7	CN

158	Leis	Amy	Golder Associates Ltd.	202 - 2174 Airport Road	Saskatoon, SK	S7L 6M6	CN
159	Letcher	Robert	Environment Canada	Nat. Wildl. Res. Cen., 100 Gamelin Blvd.	Hull, QC	K1A 0H3	CN
160	Letchford	Ryan	University of British Columbia	4478 West 14th Avenue	Vancouver, BC	V6R 2Y3	CN
161	Liber	Karsten	University of Saskatchewan	Toxicol. Centre, 44 Campus Dr.	Saskatoon, SK	S7N 5B3	CN
162	Lindeman	D.H.	Environment Canada	115 Perimeter Road	Saskatoon, SK	S7N 0X4	CN
163	Linton	Larry	University of Calgary	Toxicol. Centre, 2500 University Dr. NW	Calgary, AB	T2N 1N4	CN
164	Lintott	Darlene	HydroQual Laboratories Ltd.	#3, 6125 - 12 Street S.E.	Calgary, AB	T2H 2K1	CN
165	Luoma	Maire	SENTAR Consultants Ltd.	#200, 1122 - 4th Street SW	Calgary, AB	T2R 1M1	CN
166	Luxon	Lynne	Environment Canada	867 Lakeshore Road P.O. Box 5050	Burlington, ON	L7R 4A6	CN
167	MacDonald	Connie	Environment Canada	351 St. Joseph Blvd. 8th Floor	Hull, QC	K1A 0H3	CN
168	Macey	Debbie	HydroQual Laboratories Ltd.	#3, 6125 - 12 Street S.E.	Calgary, AB	T2H 2K1	CN
169	MacInnis	Gordia	Environment Canada	867 Lakeshore Road	Burlington, ON	L7R 4A6	CN
170	MacKinnon	Mike	Syncrude Canada Ltd.	9421-17 Avenue	Edmonton, AB	T6N 1H4	CN
171	Madajczuk	Anna	Nova Chemicals (Canada Ltd.)	P.O. Box 3060	Sarnia, ON	N7J 7M1	CN
172	Madill	Robert	University of Guelph	205 Waterloo Avenue	Guelph, ON	N1H 3J4	CN
173	Mah-Paulson	May	O'Connor Assoc. Environ. Inc.	1000, 639 - 5 Avenue SW	Calgary, AB	T2P 0M9	CN
174	Maj	Michelle	Canada Centre for Inland Waters	867 Lakeshore Road P.O. Box 5050	Burlington, ON	L7R 4A6	CN
175	Malley	Diane	Dept.of Fisheries and Oceans	Freshwater Institute, 501 University Cres.	Winnipeg, MB	R3T 2N6	CN
176	Manly	Ken	Klohn-Crippen Consultants	#114, 6815 - 8 Street NE	Calgary, AB	T2E 7H7	CN
177	Martel	Pierre	Pulp and Paper Research Institute	570 St. John's Boulevard	Pt. -Claire, QC	H9R 3J9	CN
178	Masson-Slogran	Deborah	Lakefield Research	185 Concession Street	Lakefield, ON	K0L 2H0	CN
179	Maxwell	Nancy	Chemex Labs Alberta Inc.	2021 - 41 Avenue NE	Calgary, AB	T2E 6P2	CN
180	Mayer	Tatiana	NWRI - Environment Canada	867 Lakeshore Road	Burlington, ON	L7R 4A6	CN
181	McCann	Janet	University of Guelph	Centre for Toxicology, Bovey Building	Guelph, ON	N1G 2W1	CN
182	McCarthy	Lynda	Dept. of Fisheries and Oceans	867 Lakeshore Road	Burlington, ON	L7R 4A6	CN
183	McCoy	Elaine	Macleod Institute	ES-1040, 2500 University Dr. NW	Calgary, AB	T2N 1N4	CN
184	McDonald	Les	BC Environment, Env. Protect.	205 Industrial Road G.	Cranbrook, BC	V1C 6H3	CN
185	McGreer	Eric	Fisheries and Oceans Canada	#327 - 555 West Hastings Street	Vancouver, BC	V6B 5G3	CN
186	McLeay	Don	McLeay Environmental Ltd.	2999 Spring Bay Road	Victoria, BC	V8N 5S4	CN
187	Mehta	Ram D.	Prairie Biological Research Ltd.	4290 91A Street	Edmonton, BC	T6E 5O2	CN
188	Merriman	John	Environment Canada	867 Lakeshore Road	Burlington, ON	L7R 4A6	CN
189	Miasek	Peter	Imperial Oil	P.O. Box 4029 Station A	Toronto, ON	M5W 1K3	CN
190	Moore	Daniel	Alberta Newsprint Company	P.O. Bag 9000	Whitecourt, AB	T7S 1P9	CN
191	Moran	Tim	Pollutech Enviroquatics Ltd.	704 Mara Street	Pt. Edward, ON	N7V 1X4	CN
192	Moul	David	Pacific Environ. Sci. Centre	2645 Dollarton Hwy.	N. Vancouver, BC	V7H 1V2	CN
193	Munkittrick	Kelly	Environment Canada	867 Lakeshore Road	Burlington, ON	L7R 4A6	CN
194	Munro	Scott	Lambton Industrial Society	Suite 111, 265 N. Front Street	Sarnia, ON	N0N 1G0	CN
195	Munteanu	Tina	G3 Consulting Ltd.	1A - 12880 Bathgate Way	Richmond, BC		CN
196	Murdoch	Mary	J. Whitford Environment Ltd.	607 Torbay Road	St. John's, NF	A1A 4Y6	CN
197	Murphy	George	Environmental Protection	6th Floor 9820 - 106 Street	Edmonton, AB	T5K 2J6	CN
198	Murphy	Clair	PEI Dept. of Environ. Res.	P.O. Box 2000	Charlottetown, PE	C1A 7N8	CN
199	Nagendran	Jay	Alberta Environmental Protection	Oxbridge Place, 9820 - 106 Street	Edmonton, AB	T5K 2J6	CN
200	Naish	Valerie	Domtar Inc.	22025 Trans Canda Hwy.	Senneville, QC	H9X 3L7	CN
201	Nash	Geoff	Alberta Environmental Protection	Chem. Assess. Mgmt. Div., 200 - 5th Ave. S	Lethbridge, AB	T1J 4C7	CN
202	Nason	Ted	Alberta Environmental Protection	200 5th Avenue South	Lethbridge, AB	T1J 4C7	CN
203	Ng	Clement	Alberta Environmental Protection	9820 - 106 Street	Edmonton, AB	T5K 2J6	CN
204	Niimi	Arthur	Dept. of Fisheries and Oceans	Canada Centre for Inland Waters	Burlington, ON	L7R 4A6	CN
205	Nix	Peter	Golder Associates Ltd.	500 - 4260 Still Creek Drive	Burnaby, BC	V5L 6C6	CN
206	Noton	Leigh	Alberta Environmental Protection	Water Sci. Br., Oxbridge Place, 9820 - 106 St.	Edmonton, AB	T5K 2J6	CN
207	O'Brien	Graham	University of Guelph	Centre for Toxicology	Guelph, ON	N1G 2W1	CN
208	O'Connor	Brian	Paprican	570 St. John's Boulevard	Pt. Claire, QC	H9R 3J9	CN
209	Oberly	Gail	Microbics Corp.	2232 Rutmerlord rd.	Carlsbad, CA	92008	USA
210	Oishi	Courtney	Komex International Ltd.	#100, 4500 - 16 Avenue NW	Calgary, AB	T3B 0M6	CN

211	Omotani	Ken	Alcan Smelters & Chemicals Ltd.	P.O. Box 1800	Kitimat, BC	V8C 2H2	CN
212	Orr	Patti	BEAK Consultants Ltd.	Suite 212, 260 East 16th St.	Brampton, ON	L6T 5B7	CN
213	Osborne	Jim	Environment Canada	Hazardous Waste Branch	Ottawa, ON	K1A 0H3	CN
214	Otico	P.-F.	Environ. Manag. Bureau - Phillipines	Attn: Linda c/o EVS Consultants	N. Vancouver, BC	V7P 2R4	CN
215	Paasivirta	Jaako	University of Jyvaskyla	P.O. Box 35 FIN - 40351	Jyvaskyla		Finland
216	Paine	Michael	Paine, Ledge & Associates	Suite 212, 260 East 16th St.	N. Vancouver, BC	V7L 2G9	CN
217	Pammer	Cindy		3135B - 40 Street SW	Calgary, AB	T3E 3J9	CN
218	Parker	Roy	Environment Canada	Queen Square, 45 Alderney Dr.	Dartmouth, NS	B2Y 2N6	CN
219	Parks	John	Ontario Min. Environ. Energy	435 James Street South	Thunder Bay, ON	P7E 6S7	CN
220	Parrott	Joanne	Environment Canada	867 Lakeshore Road	Burlington , ON	L7R 4A6	CN
221	Penney	Kathy	J. Whitford Environment	607 Torbay Road	St. John's, NF	A1A 4A6	CN
222	Peters	Kerry	CXY Chemicals	1500, 635 - 8th Avenue SW	Calgary, AB	T2P 3Z1	CN
223	Peters	Lisa	University of Waterloo	Biology Department	Waterloo, ON	N2L 3G1	CN
224	Pica Granados	Yolanda	Inst. Mexi. de Tecnol. Agva	Poseo Cuahuna. 8532 Progreso	Jiptepec, Morelas	62550	Mexico
225	Pickard	Janet	B.C. Research Inc.	3650 Westbrook Mall	Vancouver, BC	V6S 2L2	CN
226	Playle	Richard	Wilfrid Laurier University	Department of Biology	Waterloo, ON	N2L 3C5	CN
227	Porter	Ed	Environment Canada	351 St. Joseph Blvd.	Hull, QC	K1A 0H3	CN
228	Power	John	Environment Canada	224 W. Esplanade	N. Vancouver, BC	V7M 3H7	CN
229	Prairie	Robert	Noranda Technology Centre	240 Hymus Boulevard	Pt. Claire, QC	H9R 1G5	CN
230	Pyle	Greg	University of Saskatchewan	Dept. of Biology	Saskatoon, SK	S7N 5B3	CN
231	Raduy	Jerry	Norwest Labs	9938 - 67 Avenue	Edmonton, AB	T6E 0P5	CN
232	Ralitsch	Milan	Enviro-Test Laboratories	9936 - 67 Avenue	Edmonton, AB	T6E 0P5	CN
233	Ramsay	Brenda		321 - 215 Oswego Street	Victoria, BC	B8B 2B5	CN
234	Redenbach	Alan	Environment Canada	224 West Esplanade	N. Vancouver, BC	V7M 3H7	CN
235	Reimer	Gerry	Reimer Analytical & Assoc. Inc.	1523 West 3rd. Avenue	Vancouver, BC	V6J 1J8	CN
236	Reish	Donald	Cal. State Univ., Long Beach	Department of Biology	Long Beach, CA	90840-3702	USA
237	Rembish	Mark	University of Guelph	P.O. Box 48-7112	Guelph, ON	N1G 2W1	CN
238	Renault	Sylvie	Universtiy of Alberta .	4 - 42 Earth Sciences Bdg.	Edmonton, AB	T6G 2E3	CN
239	Renoux	Agnes	Biotechnology Research Institute	6100 Royalmount Ave.	Montreal, QC	H4P 2R2	CN
240	Richards	Jeff	Wilfrid Laurier University	Department of Biology	Waterloo, ON	N2L 3C5	CN
241	Riebel	Phil	BEAK Consultants Ltd.	455 Fenelon Suite 104	Montreal, QC		CN
242	Robbins	Jeff	HydroQual Laboratories Ltd.	#3, 6125 - 12 Street S.E.	Calgary, AB	T2H 2K1	CN
243	Robinson	Richard	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
244	Rodrigue	Danielle	CANMET - NRCan	555 Booth Street	Ottawa, ON	K1A 061	CN
245	Roest	Nellie	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
246	Rokosh	David	Ont. Min.Environ. Energy	125 Resources Rd.	Etobicoke, ON	M9P 3V6	CN
247	Romenko	Wade	Environment Canada	Box 370	Yellowknife, NT	X1A 2N3	CN
248	Rosaasen	Arden	University of Saskatchewan	610 McPherson Avenue	Saskatoon, SK	S7N 0X6	CN
249	Rousseaux	Colin	GlobalTox International Consultants Inc.	301 Melcalfe Street, #25	Ottawa, ON	K2P 1R9	CN
250	Rowsell	Andrew	Alcan Smolters & Chemicals	P.O. Box 1800	Kitimat, BC	V8C 2H2	CN
251	Ruben	H.-D.	Mexican Inst. of Water Technol.	Paseo Cuauhna., 8532, Progreso	Jiutepec Morelas		Mexico
252	Russell	Ron	University of Windsor	Great Lakes Inst. Environ. Res.	Windsor, ON	N9B 3P4	CN
253	Rutherford	Les	Environment Canada	Queens Square, 45 Alderney Dr.	Darthmouth, NS	B2Y 2N6	CN
254	Salahub	Bob	Norwest Labs	9938 - 67 Avenue	Edmonton, AB	T6E 0P5	CN
255	Salazar	Sandra	EVS Consultants	200 W Mercer Street Suite 403	Seattle, WA	98119	CN
256	Salazar	Michael	Applied Biomonitoring	11648 - 72nd Place NE	Kirkland, WA	98034	CN
257	Samek	Michaela	Envirotest - Man. Technol. Cent.	1063 Garwood Avenue	Winnipeg, MB		CN
258	Sander	Bettina	EVS Environment Consultants	195 Pemberton Avenue	N. Vancouver, BC	V7P 2R4	CN
259	Schick	Karen	Norwest Labs	9938 - 67 Avenue	Edmonton, AB	T6E 0P5	CN
260	Schryer	Richard	Golder Associates Ltd.	202 - 2174 Airport Drive	Saskatoon, SK	S7L 6M6	CN
261	Scroggins	Rick	Environment Canada	3439 River Road South	Gloucester, ON	K1A 0H3	CN
262	Servos	Mark	Environment Canada	867 Lakeshore Road	Burlington, ON	L7R 4A6	CN
263	Sevigny	James	Komex International Ltd.	Suite 100, 4500 - 16th Avenue NW	Calgary, AB	T3B 0M6	CN

264	Sferrazza	John	Aquatic Sciences Inc.	45 Hannover Drive	St. Catharine, ON	L2W 1A3	CN
265	Shaw	Randy	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
266	Shaw	Patrick	Environment Canada	200 - 1200 West 73rd Avenue	Vancouver, BC	V6P 6H9	CN
267	Shaw	Jackie	Alberta Environmental Protection	2nd Floor 2938 - 11 Street NE	Calgary, AB	T2E 7L7	CN
268	Sheiast	Bob	SENTAR Consultants Ltd.	#200, 1122 - 4th Street SW	Calgary, AB	T2R 1M1	CN
269	Shelly	Neil	Alberta Forest Prod. Assoc.	#200 - 11738 Kingsway Avenue	Edmonton, AB	T5G 0X5	CN
270	Shergill	Ranju	Alpine Environmental Ltd.	101, 3740E - 11A Street NE	Calgary, AB	T2E 6M6	CN
271	Shriner	Gillian	Aquatic Sciences Inc.	45 Hannover Drive	St. Catharine, ON	L2W 1A3	CN
272	Silva	Adolfo	Petro Canada Corporate Services	#2060 PCCW, P.O. Box 2844	Calgary, AB	T2P 3E3	CN
273	Singh	Nand	CHEMEX Labs Alberta Inc.	9331 - 48 Street	Edmonton, AB	T6B 2R4	CN
274	Skewchuk	Paul	Alberta Environmental Protection	9820 - 106 Street	Edmonton, AB	T5K 2J6	CN
275	Smith	Gary	Alberta Newsprint	Bag 9000	Whitecourt, AB	T7S 1P9	CN
276	Smits	Judit	University of Saskatchewan	Dep .of Veterinary Pathology	Saskatoon, SK	S7N 5B4	CN
277	Spafford	Mark	Alberta-Pacific Forest Ind. Inc.	Box 8000	Boyle, AB	T0A 0M6	CN
278	Sprague	John	Sprague Associates Ltd.	474 Old Scott Road	Salt Sp. Island, BC	V8K 2L7	CN
279	Sproull	Jim		5320 - 122 Street	Edmonton, AB		CN
280	Spry	Douglas	Environment Ontario	2 St. Clair Avenue West 12th floor	Toronto, ON	M4V 1L5	CN
281	Stanton	Doug	Alberta Environmental Protection	2938 - 11 Street NE	Calgary, AB	T2E 7L7	CN
282	Steeves	Lloyd	Weyerhaeuser Canada Ltd.	Postal Bag 1020	Grande Prairie, AB	T8V 3A9	CN
283	Steinback	Brian	Abitibi-Price Inc.	207 Queens Quay W., Suite 680	Toronto, ON	M5J 2P5	CN
284	Stephenson	Gladys	University of Guelph	Dept. of Environmental Biology	Guelph, ON	N1G 2W1	CN
285	Slogran						CN
286	Stroh	Jenni	Environment Canada	224 West Esplanade	N. Vancouver, BC	V7M 3H7	CN
287	Strong-Duffin	Kathy	City of Calgary - Environment Office	P.O. Box 2100, Station M	Calgary, AB		CN
288	Stuthridge	Trevor	N. Z. Forestry Research Inst. Ltd.	Private Bag 3020	Rotorua		N.Z.
289	Sunahara	Geoffrey	BRI	6100 Royalmount Avenue	Montreal, QC	H4P 2R2	CN
290	Swain	L.G.	B.C. Ministry of Environment	765 Broughton Street	Victoria, BC	V8V 1X5	CN
291	Swanson	Stella	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
292	Tache	Michel	Environment Canada	351 St. Joseph Blvd. 8th Floor	Hull, QC	K1A 0H3	CN
293	Tanner	Jim	Acres International Ltd.	500, 10201 Southport Road SW	Calgary, AB	T2W 4X9	CN
294	Taylor	Ken	Environment Canada	351 St.-Joseph Blvd. PVM	Hull, QC	K1A 0H3	CN
295	Taylor	Darrell	Nova Scotia Dept. of Environ.	P.O. Box 2167	Halifax, NS	B3E 3B7	CN
296	Thellen	Claude	Min. de L'Environnement	360 rue Franquet bur. 40	Sainte-Foy, QC	G3P 4N3	CN
297	Thoman	Greg					CN
298	Thomas	Gregory P.	G3 Consulting Ltd.	1A-12880 Bathgate Way	Richmond, BC		CN
299	Treissman	Deirdre	Radian International Canada Inc.	1610, 734 - 7th Avenue SW	Calgary, AB	T2P 3P8	CN
300	Trottier	Sylvain	Environnement Canada	105 McGill Street	Montreal, QC	H2Y 2E7	CN
301	Ulan	Julie	Alpine Environmental Ltd.	Box 6057	Drayton Valley, AB	T7A 1R6	CN
302	Van Coillie	Raymond	Environment Canada	105 McGill Street	Montreal, QC	H2Y 2E7	CN
303	Van Meer	Terry	Syncrude Canada Ltd.	P.O. Bag 4009 Drop Point 0078	Ft. McMurray, AB	T9K 3L1	CN
304	Van Roodselaar	Albert	Daishowa-Marubeni Inter. Ltd.	Bag 2200	Peace River, AB	T8S 1Y4	CN
305	Van Velzen	Kevan	Dept. of National Defence	Base Construction Eng., CFB Calgary	Calgary, AB	T3E 1T8	CN
306	Vanden Heuvel	Mike	University of Waterloo	Department of Biology	Waterloo, ON	N2L 3G1	CN
307	Vasiliauskas	Julian	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
308	Verbeek	Allen	CHEMEX Labs Alberta Inc.	9331 - 48 Street	Edmonton, AB	T6B 2R4	CN
309	Visser	Suzanne	University of Calgary	Dep. of Biological Sciences	Calgary, AB	T2N 1N4	CN
310	Walsh	Narcissus	Jacques Whitford Environ. Ltd.	607 Torbay Road	St. John's, NF	A1A 4Y6	CN
311	Watson	Tom	Golder Associates Ltd.	500-4260 Stell Creek Drive	Burnaby, BC	V5C 6C6	CN
312	Wayland	Mark	Environment Canada	Wildlife Centre, 115 Perimeter Road	Saskatoon, SK	S7N 9O4	CN
313	Webster	Gary	C.A.P.P.	2100, 350 - 7th Avenue SW	Calgary, AB		CN
314	Weeks	Jason	Inst. Terres. Ecol., Monks Wood	Abbots Ripton, Huntingdon	Cambridgeshire	PE17 2LS	U.K.
315	Wells	Peter	Environment Canada	45 Alderney Drive	Dartmouth, NS	B2Y 2N6	CN
316	Whiteway	Sandra	Jacques Whitford Environment	607 Torbay Road	St. John's, NF	A1A 4Y6	CN

317	Whitley	Gerry	DIAND Water Resources	345 - 300 Main Street	Whitehorse, YK	Y1A 2B5	CN
318	Whitney	Phil	Weldwood of Canada Ltd.	760 Switzer Drive	Hinton, AB	T7V 1V7	CN
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320	Wilairatanadilok	W.	Pollution Control Dept. - Thailand	Attn: Linda c/o EVS Consultants	N. Vancouver, BC	V7P 2R4	CN
321	Wilkes	Brian	CCME	Suite 400 - 326 Broadway Avenue	Winnipeg, MB	R3C 0S5	CN
322	Williams	David	O'connor Associates	1000 639 5th ave sw	Calgary, AB	T2P 0M9	CN
323	Williamson	Dwight	Manitoba Environment	Bldg. 2, 139 Tuxedo Avenue	Winnipeg, MB	R3N 0H6	CN
324	Wilson	Jeff	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
325	Wilson	Anne	Environment Canada	Box 370	Yellowknife, NT	X1A 2N3	CN
326	Wilson	Jonatham	Morgan State University	1700 Cold Spring Lane, CGW236	Baltimore, MD	21239	USA
327	Wilson	Ross	CanTox Inc.	666 Barrard Street Suite 1300	Vancouver, BC		CN
328	Windle	Wiletta	Environment Canada	351 St.-Joseph Blvd., PVM	Hull, QC	K1A 0H3	CN
329	Wong	Mike	Environment Canada	351 St. Joseph, Blvd., PVM	Hull, QC	K1A 0H3	CN
330	Wood	Craig	Noranda Technology Centre	240 Hymus Boulevard	Pt. Claire, QC	H9R 1G5	CN
331	Yang	Jason	Golder Associates Ltd.	1011 - 6th Avenue SW	Calgary, AB	T2P 0W1	CN
332	Yee	Stewart	Environment Canada	2645 Dollarton Highway	N. Vancouver, BC	V7H 1V2	CN
333	York	Roberta	Inspection Branch - DFO	501 University Crescent	Winnipeg, MB	R3T 2N6	CN
334	Young	Leslie	Hatfield Consultants Ltd.	201, 1571 Bellevue Avenue	W. Vancouver, BC		CN
335	Zajdlik	Barry	Zajdlik Associates	R.R. #3	Rockwood, ON	N0B 2K0	CN
336	Zemanek	Mike	Alberta Environmental Protection	Oxbridge Place, 9820 - 106 St.	Edmonton, AB	T5K 2J6	CN
337	Zitko	Vlado	Dept. of Fisheries and Oceans	St. Andrews Biological Station	St. Andrews, NB	E0G 2X0	CN

ERRATUM

The "Proceedings of the 22nd Annual Aquatic Toxicity Workshop: October 2-4, 1995, St. Andrews, New Brunswick," K. Haya and A.J. Niimi (Editors), Can. Tech. Rep. Fish. Aquat. Sci. 2093: xi + 160 p., inadvertently reported an error in the citation of the paper by Starodub et al. on page 138.

The correct title for this paper should have read:

METHODOLOGY FOR RISK ASSESSMENT OF PETROLEUM HYDROCARBON CONTAMINATION IN THE AQUATIC ENVIRONMENT. M.E. Starodub[1], C.E. Moore[2], L.C. Mucklow[2] and R.F. Willes[1]. [1]CanTox Inc., 2233 Argentia Rd., Suite 308, Mississauga, ON L5N 2X7; [2]CanTox Inc., 5475 Spring Garden Rd., Suite 603, Halifax, NS B3J 3T2.

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER

The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings are available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

Proceedings of the 22nd Annual Aquatic Toxicity Workshop: October 2-4, 1995, St. Andrews, New Brunswick. Edited by K. Haya and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2093: 159 p.

Proceedings of the 21st Annual Aquatic Toxicity Workshop: October 3-5, 1994, Samia, Ontario. Edited by G.F. Westlake, J.L. Parrott and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2050: 179 p.

Proceedings of the 20th Annual Toxicity Aquatic Workshop: October 17-21, 1993, Quebec City, Quebec. Edited by R. Van Coillie, Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel and C. Thellen. Can. Tech. Rep. Fish. Aquat. Sci. 1989: 331 p.

Proceedings of the 19th Annual Aquatic Toxicity Aquatic Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore. Can. Tech. Rep. Fish. Aquat. Sci. 1942: 489 p.

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